

**The Effects of Glycaemic Perturbations on Cognitive and
Vascular Haemodynamic Functions in
Adult Humans**

By

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**To Tasmin, for your unyielding support, and to Charlie and Emily, for making
me smile.**

Declaration

I declare that:

I have composed this thesis in its entirety.

The thesis has not been accepted in any previous application for a degree.

I have performed the work myself, and while assisted by several colleagues in data collection, the data analysis and critical interpretation are entirely my own work.

The sources of all information are acknowledged and referenced.

Signed:

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Abstract of Thesis

Diabetes is a syndrome caused by lack of insulin and is characterised by hyperglycaemia and deranged metabolism. The non-physiological doses of insulin that are used in standard treatment regimens often leads to a mismatch between blood glucose and plasma insulin concentrations, resulting in hypoglycaemia. The human brain is dependent upon glucose as its main source of energy and rapidly malfunctions if deprived of this substrate. Controlled hypoglycaemia can be induced experimentally using the hyperinsulinaemic glucose clamp technique and this method can be used to examine the effects of acute hypoglycaemia on cognitive performance. In general, tests that involve attention, concentration, psychomotor skill and the ability to ignore distracting information tend to deteriorate when blood glucose declines below about 3.0 mmol/L. Memory, the cognitive process of storing, encoding and retrieving information, is one of the most crucial domains of cognition, yet memory function has seldom been examined during acute hypoglycaemia, and the few studies that have attempted to do this have provided inconsistent results.

To protect the function and integrity of the central nervous system from hypoglycaemia and restore homeostasis as rapidly as possible, a hierarchy of responses are activated when blood glucose falls below 4.0 mmol/l. The rise in blood glucose is mediated by the secretion of counterregulatory hormones, of which adrenaline is one of the major components. Sympatho-adrenal stimulation provokes significant haemodynamic changes, including an increased heart rate and stroke volume, increased myocardial contractility and a rise in cardiac output. In addition, peripheral blood pressure is affected with an increase in systolic and decrease in diastolic pressure, while mean arterial pressure remains unchanged. Pressure values

in the peripheral circulation are an inaccurate measure of central pressure because of amplification of the pressure pulse between central and peripheral arteries. Central systolic and diastolic pressures in the aorta are determinants of cardiac loading and perfusion and directly influence cardiac function.

The aims of this research project were to examine the effects of acute hyperglycaemia on cognitive function and mood, the effects of acute hypoglycaemia on memory function, and to examine the central haemodynamic responses to acute hypoglycaemia.

Study 1

The effects of experimentally-induced acute moderate hypoglycaemia on memory function will be examined in 16 non-diabetic humans. It is hypothesised that memory function will be significantly impaired during hypoglycaemia. Each subject will participate in two laboratory sessions, representing two different experimental conditions, that will be separated by at least 2 weeks. A modified hyperinsulinaemic glucose clamp will be used to maintain the blood glucose at a predetermined level. In the euglycaemia condition, the arterialised blood glucose concentration was maintained at 4.5 mmol/L and hypoglycaemia will not be induced. In the hypoglycaemia condition, the glucose concentration will be lowered to 2.5 mmol/L. The subjects will not be informed which experimental arm of the study is being performed on each occasion and will undergo the two experimental sessions in a randomised and counterbalanced fashion. Validated tests of immediate and delayed verbal memory, immediate and delayed visual memory, and tests of working memory will be administered during the study conditions. A general linear model

(repeated measures analysis of variance) will be used to analyse the results with order of session (euglycaemia-hypoglycaemia or hypoglycaemia-euglycaemia) as a 'between subjects' factor, and condition (euglycaemia or hypoglycaemia) as a 'within subjects' factor. A p value < 0 .05 will be considered to be significant, and effect size will be calculated using Eta squared.

Study 2

An identical study design will be used to assess memory function during acute hypoglycaemia in 16 young adults with type 1 diabetes. Identical methods will be used to assess this separate group and the hypothesis is the same. Subjects recruited for the study will not have any clinical evidence of microvascular disease, will have a short duration of diabetes (< 10 years) and will have normal symptomatic awareness of hypoglycaemia.

Study 3

Cognitive function and mood will be examined during acute hyperglycaemia in 20 subjects with type 2 diabetes. A hyperinsulinaemic glucose clamp will be used to precisely control the arterialised blood glucose concentration. Two studies will be performed at two week intervals. In one study, the arterialised blood glucose concentration will be maintained at 4.5 mmol/l (euglycaemia), and in the other study hyperglycaemia will be induced (16-19 mmol/l). Studies will be performed in a randomised and counterbalanced fashion, and the subjects will be blinded to their blood glucose concentration at all times. A broad battery of cognitive tests, including tests of memory and attention, will be administered during the studies. A

mood questionnaire will also be administered. It is hypothesised that hyperglycaemia will be associated with impairment of cognitive function and change in mood state.

Study 4

The central haemodynamic effects of acute insulin-induced hypoglycaemia will be examined in 10 non-diabetic subjects. Pulse wave analysis allows the measurement of central arterial pressure and the degree of its augmentation by pulse wave reflection to be studied reliably and non-invasively using applanation tonometry, with the external application of a micromanometer-tipped probe to record peripheral pulse waveforms. The radial artery is usually used for these measurements because of its close proximity to, and support provided by, nearby bony structures. The pressure contour changes as it travels from the aorta to more peripheral sites so that pressures at the radial artery cannot be used directly as a surrogate for central pressure in the aorta. However, it is possible to estimate the central aortic pressure wave from measurements of radial artery tonometry with the use of a mathematical transformation. Acute hypoglycaemia will be induced by a continuous intravenous infusion of insulin. The infusion will be continued until the onset of the autonomic reaction associated with the induction of hypoglycaemia. The autonomic reaction is identified as an abrupt rise in heart rate (15% above baseline), the rapid onset of symptoms of hypoglycaemia (such as sweating and tremor), and an increase in systolic blood pressure as measured peripherally. As soon as the autonomic reaction has been reached hypoglycaemia will be reversed to restore normoglycaemia within 30 minutes. Serial measurements of radial artery pressure will be recorded by applanation tonometry using a hand held tonometer. Heart rate will be monitored

continuously throughout the study using precordial electrodes. Peripheral blood pressure will be recorded every 10 minutes using a digital automated sphygmomanometer.

Study 5

A similar study design will be used to examine the central haemodynamic responses to insulin-induced hypoglycaemia in ten subjects with short duration (< 10 years) type 1 diabetes.

Study 6

A further study, using an identical study design, will be used to measure the central haemodynamic changes in response to acute hypoglycaemia in ten subjects with longer duration (>15 years) type 1 diabetes.

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Summaries of Studies

Study 1

Aim: To examine the effects of acute insulin-induced hypoglycaemia on short-term-, delayed- and working memory in healthy adults.

Research Design and Methods: A hyperinsulinaemic glucose clamp was used to maintain arterialised blood glucose at either 4.5 (euglycaemia = E) or 2.5 (hypoglycaemia = H) mmol/l on two occasions in 16 non-diabetic human volunteers. Tests of immediate and delayed verbal memory, immediate and delayed visual memory and working memory were administered during each experimental condition. Two tests of general cognitive ability, the Trail Making B and Digit Symbol tests, were also administered.

Results: Immediate verbal memory was assessed by the Auditory Verbal Learning Test (AVLT) and Logical Memory test. Scores deteriorated from a mean (SD) of 43.6 (7.0) and 27.7 (5.9) during E to 33.9 (7.7) ($p=0.001$) and 24.4 (5.0) ($p=0.004$) during H, respectively. The Benton Visual Retention Test, a test of immediate visual memory, deteriorated from 6.3 (1.7) (E) to 5.3 (1.6) (H) ($p=0.041$). The effect of hypoglycaemia on working memory was very powerful. Four-term Order deteriorated from 11.5 (4.9) (E) to 2.5 (1.9) (H) ($p<0.0001$). Three other tests of working memory showed an equivalent degree of decrement during H. Delayed memory, assessed by AVLT Delayed, deteriorated from 10.3 (2.1) (E) to 4.5 (1.6) (H) ($p<0.0001$). Logical Memory Delayed deteriorated from 15.1 (4.0) (E) to 8.1 (2.9) (H) ($p=0.001$) and Visual Reproduction Delayed deteriorated from 21.5 (16.5) (E) to 7.6 (5.5) (H) ($p=0.001$). Performance in the Trail Making B and Digit Symbol

tests deteriorated from a mean (SD) of 41.5 (7.9) seconds and 70.1 (13.0) during E to 62.6 (11.0) seconds ($p<0.0001$) and 59.6 (14.4) ($p=0.001$) during H respectively.

Conclusions: All memory systems are impaired during acute hypoglycaemia, with working memory and delayed memory being particularly susceptible. These findings are of importance to people with insulin-treated diabetes, in whom mild (self-treated) hypoglycaemia is common, and in whom such effects of hypoglycaemia on memory will interfere with many daily activities.

Study 2

Aim: To examine the effects of acute insulin-induced hypoglycaemia on short-term, delayed and working memory in people with type 1 diabetes.

Research Design and Methods: A hyperinsulinaemic glucose clamp was used to maintain arterialized blood glucose at either 4.5 (euglycaemia = E) or 2.5 (hypoglycaemia = H) mmol/l on two separate occasions in 16 adults with type 1 diabetes. The participants completed tests of immediate and delayed verbal memory, immediate and delayed visual memory, and working memory during each experimental condition. Two other mental tests, the Trail Making B and Digit Symbol tests, were also administered.

Results: Performance in tests of immediate verbal and immediate visual memory was significantly impaired during hypoglycaemia. The effect of hypoglycaemia on working memory and delayed memory was more profound. Performance in the non-memory tests, the Trail Making B and Digit Symbol Tests, also deteriorated during hypoglycaemia.

Conclusions: All of the memory systems examined in the present study were affected significantly by acute hypoglycaemia, particularly working memory and delayed memory. As mild (self-treated) hypoglycaemia is common in people with insulin-treated diabetes, these observed effects of hypoglycaemia on memory are of potential clinical importance, as they could interfere with many everyday activities.

Study 3

Aim: To examine the effects of acute hyperglycaemia on cognitive function and mood in people with type 2 diabetes.

Research Design and Methods: 20 subjects with type 2 diabetes (median (range) aged 61.5 (53.1-72.0) years with Body Mass Index 29.8 (22.0-34.6) kg/m², duration of diabetes 5.9 (2.8-11.2) years and HbA1c 7.5 (6.7-8.4) %) were recruited to the study. A hyperinsulinaemic glucose clamp was used to maintain arterialised blood glucose at either 4.5 (euglycaemia) or 16.5 (hyperglycaemia) mmol/l on two occasions in a randomised and counterbalanced fashion. Tests of information processing, immediate and delayed memory, working memory and attention were administered with a mood questionnaire during each experimental condition.

Results: Performance in complex tests of cognitive function was impaired during acute hyperglycaemia. For example, the Four Choice Reaction Time test, a test of information processing, deteriorated from a mean (SD) score of 710 (116) milliseconds during euglycaemia to 775 (122) milliseconds during hyperglycaemia ($p < 0.0001$, $\eta^2 = 0.56$). Mood was also significantly affected during hyperglycaemia, with a decrease in energetic arousal and hedonic tone, and an increase in tense arousal.

Conclusions: Acute hyperglycaemia causes impairment of cognitive function and alteration of mood state in people with type 2 diabetes. These findings are of practical importance to people with type 2 diabetes, in whom mild hyperglycaemia is common, and in whom such effects of hyperglycaemia on cognitive function and mood may interfere with many daily activities.

Studies 4, 5 and 6

Aim: To examine the effects of intravenous insulin and acute hypoglycaemia on arterial wall stiffness and central haemodynamic responses in adults with and without type 1 diabetes.

Research Design and Methods: In thirty young male volunteers (10 non-diabetic; 10 with type 1 diabetes <5 years duration; 10 with type 1 diabetes >15 years duration) intravenous insulin was administered to provoke an acute autonomic reaction (R) to hypoglycemia. Heart rate, peripheral blood pressure and pulse wave analysis (radial artery) were monitored. Augmentation index (AIx), a measure of arterial wall stiffness and wave reflection, and central arterial pressure were recorded.

Results: At baseline no significant differences were observed between Groups 1 and 2 in either AIx or in central arterial pressure but in Group 3 both measures were significantly higher. All three groups exhibited similar responses to intravenous infusion of insulin and to hypoglycaemia: AIx fell progressively from baseline to R, peripheral systolic blood pressure increased, while central systolic pressure decreased

Conclusions: When compared to age- and sex-matched non-diabetic controls, people who had type 1 diabetes of long duration had increased stiffness of vessel walls. The opposing responses in peripheral and central systolic pressures during hypoglycaemia may be related to the reduction in AIx, which causes diminished amplification of the systolic pressure wave. Changes in AIx are probably mediated by a direct action of insulin on arterial endothelium, or changes in heart rate. These

functional changes may contribute to the increased cardiovascular morbidity that is associated with type 1 diabetes of long duration.

PART I

BACKGROUND

Chapter 1

Pathophysiological and Clinical Aspects of Hypoglycaemia

INTRODUCTION

Hypoglycaemia is a common side-effect of treatment with insulin and affects most people with insulin-treated diabetes (1). Hypoglycaemia is also associated with sulphonylurea therapy, but occurs much less frequently. Physiological insulin secretion requires transient peaks of plasma insulin associated with meals and with a relatively constant and low basal level of insulin secretion during periods of fasting. Most current methods of insulin replacement therapy for people with diabetes are far from physiological, and intra- and inter-individual absorption of insulin is highly variable. Despite improvements in insulin formulations, methods of insulin delivery and treatment regimens, fluctuations in blood glucose are common and it remains difficult to match the administration of exogenous insulin to an individual's energy requirements on a daily basis. Occasional and unpredictable hypoglycaemia is therefore almost an inevitable consequence of effective insulin therapy. Hypoglycaemia can be extremely unpleasant, is associated with acute and chronic morbidity (2), and can impinge on every aspect of everyday life. Severe hypoglycaemia is greatly feared and is the major limiting factor to achieving and maintaining good glycaemic control.

The Diabetes Control and Complications Trial (DCCT) (3) and the Stockholm Diabetes Intervention Study (SDIS) (4) in type 1 diabetes, and the UK Prospective Diabetes Study (UKPDS) (5) in type 2 diabetes evaluated the effect of strict glycaemic control on the incidence and progression of diabetic complications and documented the frequency of adverse effects of treatment. These studies have shown unequivocally that strict glycaemic control limits the development and progression of diabetic

microangiopathy, but is accompanied by a higher rate of severe hypoglycaemia, particularly in type 1 diabetes. Current therapeutic policies that attempt to achieve continuous normoglycaemia with intensive insulin regimens, and the increasing use of insulin at an earlier stage of management in type 2 diabetes, are likely to promote more frequent hypoglycaemic events of varying severity. It is important that physicians recognise the potential risks associated with hypoglycaemia, as deliberate avoidance of this problem influences modern therapeutic strategies for the clinical management of diabetes.

MAINTENANCE OF NORMOGLYCAEMIA IN HUMANS

The human brain is dependent upon glucose as its main source of energy and rapidly malfunctions if deprived of this substrate. To protect the function and integrity of the central nervous system and restore homeostasis as quickly as possible, a hierarchy of responses are activated when the blood glucose concentration falls (6-10).

Continuous adjustment of blood glucose is controlled through a blood glucose sensor mechanism in the portal circulation, with central feedback to the brain, which modifies insulin secretion (11). This system responds to, and controls, the normal physiological fluctuations in blood glucose in response to the ingestion of food and the energy demands of exercise, but is unable to cope with a significant decline in blood glucose associated with the hyperinsulinaemia caused by administration of exogenous insulin. Insulin inhibits hepatic and renal glucose production, and stimulates glucose

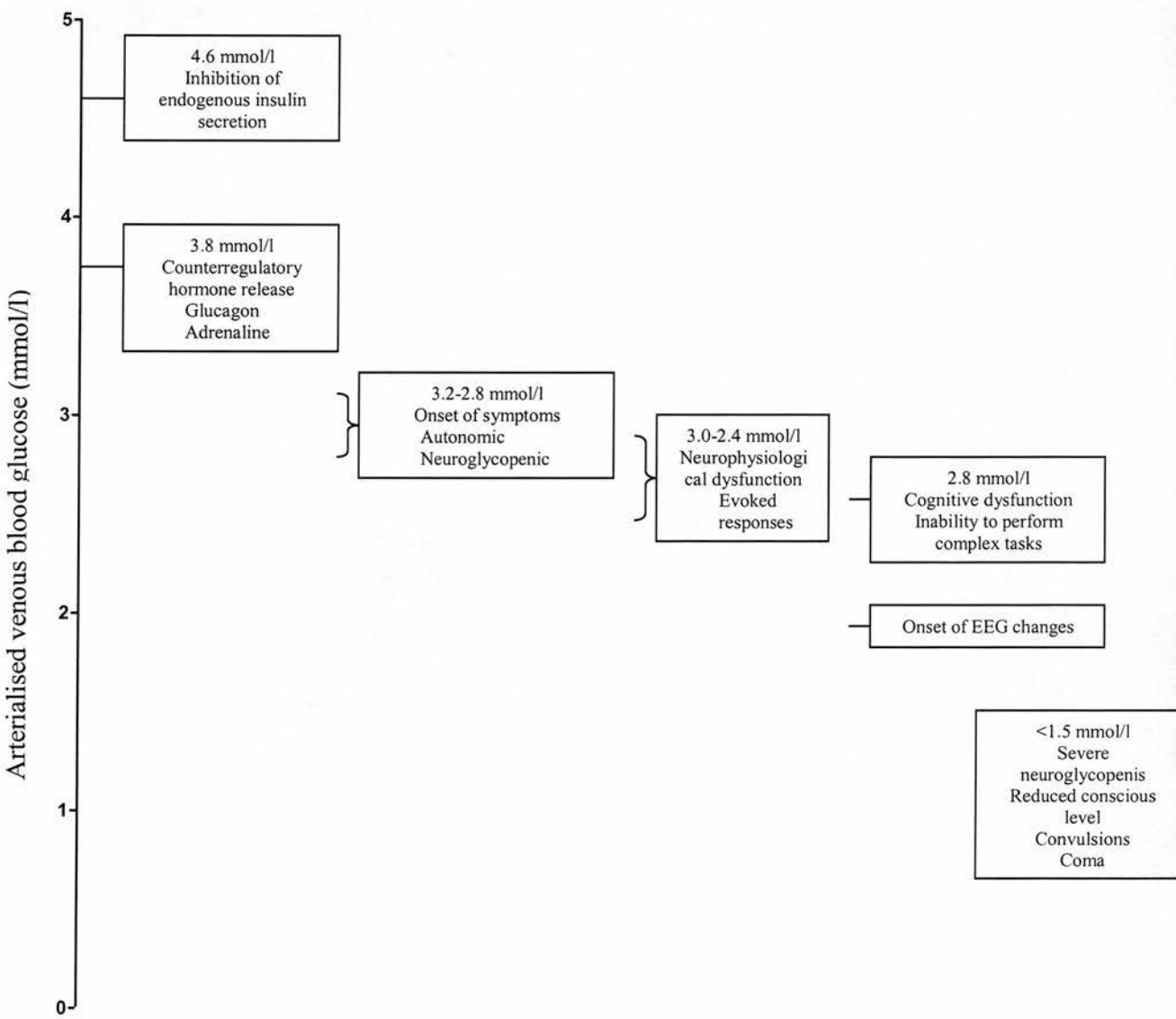
uptake and utilisation by peripheral tissues, especially skeletal muscle, causing a fall in blood glucose. The initial response to a decline in blood glucose is the suppression of endogenous insulin secretion, which commences at an arterialised blood glucose concentration of approximately 4.6 mmol/l, well within the normal physiological range. The subsequent prevention and correction of hypoglycaemia is dependent on the secretion of various counterregulatory hormones, the principal ones being glucagon, adrenaline, growth hormone and cortisol. Glucagon works only in the liver and stimulates hepatic glucose production, while adrenaline stimulates hepatic and renal glucose production and limits peripheral glucose utilisation. Adrenaline is not essential for the correction or prevention of hypoglycaemia, but its role becomes critical when glucagon is deficient or absent. Growth hormone and cortisol also restrict glucose utilisation and support glucose production, but have a lesser role in the hierarchy of acute counterregulatory activity as their actions occur more slowly than glucagon and adrenaline, and they have greater importance in the counterregulatory response to prolonged hypoglycaemia.

Studies in animals have indicated that counterregulatory responses are initiated by the stimulation of glucose sensors within the hypothalamus in the brain (7), in addition to the peripheral sensor mechanism in the portal vein (11), and glucopenia appears to have a direct effect on the alpha cells of the pancreatic islets to promote glucagon secretion (7). Central activation of the autonomic nervous system results in stimulation of the peripheral sympatho-adrenal system and includes a profuse secretion of adrenaline, equivalent to that observed in major medical emergencies such as acute myocardial infarction. While adrenaline has a prominent counterregulatory role, it also

augments physiological responses that occur as a consequence of autonomic arousal, such as characteristic haemodynamic changes (e.g. tachycardia) and sweating. These changes invoke subjective sensations that are interpreted by the brain as symptoms of hypoglycaemia. Thus, generation of these warning symptoms alert the individual to the potential threat that hypoglycaemia poses to the brain and so stimulate an appropriate behavioural response (the ingestion of food).

The glycaemic thresholds at which the secretion of the counterregulatory hormones commences, are just below the normal physiological range of blood glucose (arterial blood glucose concentration 3.8 mmol/l). By contrast, the glycaemic thresholds for the onset of symptoms of hypoglycaemia (3.0 mmol/l) and the development of cognitive impairment (2.7 mmol/l) occur at lower blood glucose concentrations. Glycaemic thresholds are reproducible in non-diabetic individuals (12) (Figure 1.1), but are dynamic in people with diabetes and can be altered by external factors such as very strict glycaemic control or recent preceding (antecedent) hypoglycaemia (13).

Figure 1.1: Hierarchy of endocrine, symptomatic and neurophysiological responses to acute hypoglycaemia in non-diabetic subjects. Reproduced from *Textbook of Diabetes*, 2nd edition, 1997 (eds Pickup J and Williams G), with the permission of the publishers.



SYMPTOMS OF HYPOGLYCAEMIA

The development of subjective symptoms of hypoglycaemia is fundamental to the early detection and treatment of hypoglycaemia. Perception and interpretation of the symptoms is crucial to this process, and is inherent to the concept of 'awareness' of hypoglycaemia. Symptoms are idiosyncratic with considerable individual variation in their nature and intensity in adults, and can be classified as autonomic, neuroglycopenic or non-specific (Table 1.1) (14). Autonomic symptoms, such as tremor, palpitations and sweating, are generated through activation of the sympatho-adrenal system. Because glucose is the principal energy source for the brain, hypoglycaemia rapidly affects and impairs neuronal function through neuroglycopenia, that is manifested by symptoms such as confusion, drowsiness and incoordination. The range of symptoms of hypoglycaemia is age-specific. In children features of behavioural change are common (15,16), while in the elderly neurological symptoms are prominent (17,18). Both the character and the intensity of symptoms may change over time and are affected by numerous biological and psychosocial modifiers. Factors that affect the magnitude of the physiological responses to hypoglycaemia include the depth and duration of hypoglycaemia, the rate of decline of blood glucose, and, in a person with diabetes, the duration of diabetes, the quality of glycaemic control and history of previous exposure to hypoglycaemia. The symptoms of hypoglycaemia are also modified by posture (the intensity of autonomic symptoms is greater when standing compared to lying) (19), medications, such as sedatives and beta-adrenoceptor blockers (20), and alcohol (21). Circumstances and activities can also affect the identification of symptoms. Diminished

perception of warning symptoms may be associated with the syndrome of impaired awareness of hypoglycaemia (see Chapter 1.2).

Table 1.1: Common symptoms of hypoglycaemia in humans (based on Deary *et al*, 1999)

Autonomic	Neuroglycopenic	Non-specific
Sweating	Confusion	General malaise
Palpitations	Drowsiness	Headache
Shaking	Odd behaviour	Nausea
Hunger	Speech difficulty	
	Incoordination	

CAUSES OF HYPOGLYCAEMIA

Because blood glucose excursions are very tightly controlled in the non-diabetic state, spontaneous hypoglycaemia is very rare, whereas episodic hypoglycaemia is common in people with insulin-treated diabetes (1). An episode of hypoglycaemia is classified as *mild*, if the individual is able to self-treat, and *severe* if the individual requires the assistance of another person. Severity is not classified either by the nature or intensity of the symptom response, the effect on conscious level, or the type of treatment required.

Causation is often multifactorial and no definite cause can be identified in many episodes of severe hypoglycaemia, which usually result from a mismatch between the absorption of food and insulin, and may be aggravated by a failure to perceive a symptomatic warning.

Common causes of hypoglycaemia are listed in Table 1.2. These include:

Dosage errors: In addition to failure to adjust insulin dosage appropriately for premeditated exercise or reduced or delayed intake of food, mistakes occur in the measurement of insulin doses, particularly in people with visual impairment or those with a faulty injection technique. The impact of a small error in insulin dose is much greater in young children.

Exercise: If plasma insulin concentrations are low, exercise stimulates increased hepatic glucose production and promotes hyperglycaemia. However, if they are high, hepatic glucose production and peripheral glucose utilisation are inhibited and, unless more

carbohydrate is consumed, hypoglycaemia will ensue. The absorption of insulin is accelerated if exercise commences shortly after the insulin injection (particularly when the insulin has been injected into an exercised limb, such as the leg), and enhances the risk of hypoglycaemia.

Renal Failure: Insulin requirements are lower in people whose renal function is impaired, as their metabolic clearance of insulin is reduced. People with renal impairment have a five-fold higher incidence of severe hypoglycaemia compared to matched subjects with normal kidney function (22).

Co-existent endocrine disease: Any condition which decreases the concentration of anti-insulin hormones will increase insulin sensitivity and predispose to the development of hypoglycaemia. Endocrine disorders which result in cortisol deficiency, such as Addison's disease and hypopituitarism, are associated with a higher frequency of hypoglycaemia.

Malabsorption and gastroparesis: Malabsorptive conditions, such as coeliac disease (which is associated with type 1 diabetes), and delayed gastric emptying or frank gastroparesis resulting from autonomic neuropathy, can decrease or delay carbohydrate digestion and absorption, and promote hypoglycaemia (23).

Factitious hypoglycaemia: Deliberate induction of hypoglycaemia is associated with psychological or psychiatric disorders and is often difficult to detect or confirm. It should be suspected if repeated severe hypoglycaemia occurs with no obvious cause. This problem is sometimes observed in young women who are recognised to have unstable glycaemic control, and is sometimes referred to as 'brittle' diabetes.

Table 1.2: Factors that precipitate or predispose to hypoglycaemia in people with diabetes treated with insulin or sulphonylureas

Excessive insulin concentrations		Increased insulin sensitivity	Inadequate carbohydrate intake
Excessive dosage	Increased insulin bioavailability		
Error by patient, doctor or pharmacist	Change of insulin species	Counterregulatory hormonal deficiencies	Missed, small or delayed meals
Deliberate overdose (factitious hypoglycaemia)	Insulin antibodies (release bound insulin)	Weight loss	Slimming diets; anorexia nervosa
Inappropriate insulin regimen for patient's lifestyle	Renal failure (reduced insulin clearance)	Physical training and exercise	Vomiting, including gastroparesis
Accelerated absorption <ul style="list-style-type: none">• Exercise• Change of injection site	Remission after starting insulin therapy (partial β cell recovery)	Postpartum	Breast feeding
		Menstrual cycle variation	Failure to cover exercise (early or delayed hypoglycaemia)
		Co-existing (untreated) endocrine disorders <ul style="list-style-type: none">• Addison's disease• Hypopituitarism	

Sulphonylurea therapy is also associated with hypoglycaemia (5), although it occurs less frequently than with insulin and is seldom as severe. It is commoner with many of the long-acting sulphonylureas, such as glibenclamide (24). Advanced age and fasting have been identified as major risk factors associated with sulphonylurea-induced hypoglycaemia (25), and others are listed in Table 1.3. Adverse drug interactions are recognised to occur between sulphonylureas and other commonly prescribed medications that may increase the risk of sulphonylurea-induced hypoglycaemia. In one comprehensive review, 15% of patients who had developed sulphonylurea-induced hypoglycaemia were simultaneously taking medications known to increase the risk of hypoglycaemia (25). Drugs that can potentiate the biological activity of sulphonylureas include aspirin, warfarin, non-steroidal anti-inflammatory drugs and alcohol.

The risk factors for severe hypoglycaemia in adults with insulin-treated diabetes are listed in Table 1.4, and include:

Strict glycaemic control: Within the last decade, the results of major prospective studies have confirmed that strict glycaemic control in type 1 diabetes is associated with a three-fold greater risk of hypoglycaemia (3,4,26), as the margin for error is small.

Impaired awareness of hypoglycaemia: Perception of the onset of warning symptoms of hypoglycaemia is either diminished or absent in approximately 25% of people with type 1 diabetes (27,28), but this is uncommon in people with insulin-treated type 2 diabetes (29). In this syndrome, the early onset of hypoglycaemia is not detected and a six-fold higher frequency of severe hypoglycaemia is reported (30).

Sleep: Most people do not experience symptoms of hypoglycaemia during sleep when catecholamine responses to hypoglycaemia are diminished (31), and are seldom wakened by a low blood glucose. Sleep is therefore a true physiological cause of impaired awareness of hypoglycaemia. Nocturnal hypoglycaemia is common in people treated with insulin.

Table 1.3: Risk factors associated with sulphonylurea therapy (e.g. glibenclamide, glipizide) in type 2 diabetes

<ul style="list-style-type: none">• Age (not dose)• Previous history of cardiovascular disease or stroke• Impaired renal function• Reduced food intake• Gastrointestinal disease e.g. diarrhoea• Alcohol ingestion• Interaction with other drugs
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Table 1.4: Risk factors for severe hypoglycaemia

<ul style="list-style-type: none">• Strict glycaemic control• Impaired hypoglycaemia awareness• Previous history of severe hypoglycaemia• Increasing age• Sleep• Excessive consumption of alcohol or binge drinking• Lower social class• C-peptide negativity; serum ACE concentration• Renal impairment• Special groups: extremes of age (children, elderly) pregnancy (insulin-treated patients)

FREQUENCY OF HYPOGLYCAEMIA

Type 1 diabetes

People with type 1 diabetes who attempt to achieve strict glycaemic control generally experience an undetermined number of episodes of asymptomatic biochemical hypoglycaemia; blood glucose concentrations have been estimated to be below 3.3 mmol/l around 10% of the time (32). Mild episodes of symptomatic hypoglycaemia are usually quickly forgotten by the individual and can only be recalled with accuracy for up to one week (33). The recollection of episodes of severe hypoglycaemia is more robust, but total amnesia of an event is not uncommon, and enquiries may have to be made of relatives or friends to obtain an accurate estimate.

Several studies have examined how often hypoglycaemia occurs in people with type 1 diabetes. There is some disparity between reported frequencies, which is principally related to differences in definition of severity of hypoglycaemia, heterogeneity of diabetic populations studied, the quality of glycaemic control, and accuracy of the methods of ascertainment (prospective vs. retrospective). A multicentre study that was performed in Denmark and England recorded the annual frequency of hypoglycaemia prospectively in over 1000 people with type 1 diabetes (34). Mild hypoglycaemia was common with an incidence of 2.0 episodes/patient/week, and the incidence of severe hypoglycaemia was 1.3 episodes/patient/year. The frequency of episodes of severe hypoglycaemia was noted to increase with duration of diabetes, and with a rising prevalence of impaired awareness of hypoglycaemia. These figures are similar to the results of a previous study (33), performed more than a decade earlier,

when different insulin regimens and formulations were in use. The incidence of severe hypoglycaemia was similar to those reported by other studies of unselected patient cohorts in various northern European countries (1.0-1.7 episodes/patient/year) in which the same definitions of hypoglycaemia were used (2,4,33,35,36).

In the large prospective DCCT study, a lower incidence of severe hypoglycaemia of 0.6 episodes/patient/year was recorded in the group with strict glycaemic control (26), but this was still three fold higher than the group with conventional treatment and less strict control. However, in the DCCT the participants were a highly selected and atypical group of young, healthy, motivated individuals of above average intelligence, who had type 1 diabetes of short duration, and the cohort did not include people with a history of preceding severe hypoglycaemia. German surveys which have recorded lower rates of severe hypoglycaemia (37) used a more rigorous definition for severe hypoglycaemia (coma and/or requiring parenteral therapy), whereas when the consensus definition of 'requiring external assistance' was applied, their frequency was similar to other studies (38).

These rates of severe hypoglycaemia have been derived in Western European and North American populations and may not be comparable in other parts of the world, where the frequency of insulin-treated diabetes and the treatment regimens may differ considerably.

Type 2 diabetes

Iatrogenic hypoglycaemia is widely considered to be less frequent in type 2 diabetes than type 1 diabetes (5,39,40). It is principally associated with insulin

treatment and the frequencies recorded with different treatment modalities in the UKPDS are shown in Table 1.5. Several other studies have also examined the frequency of hypoglycaemia in people with insulin-treated type 2 diabetes. A large 12-month retrospective, cross-sectional study of African Americans with type 2 diabetes revealed that mild hypoglycaemia occurred in 30% of patients treated with insulin and in 16% of those on oral hypoglycaemic agents; severe hypoglycaemia occurred only in those taking insulin (41). As is observed in type 1 diabetes, the incidence of hypoglycaemia in people with type 2 diabetes is greater with intensive therapy. However, the event rates of severe hypoglycaemia were approximately 10-fold lower in type 2 diabetes even during aggressive therapy with insulin (5). People with advanced type 2 diabetes (i.e. who are insulin-deficient) have deficient hormonal counterregulation (42), analogous to what is observed in type 1 diabetes. Hypoglycaemia becomes progressively more frequent with an increase in duration of insulin-treatment for type 2 diabetes, and when they are matched for duration of insulin therapy the rates of severe hypoglycaemia begin to approach those recorded in people with type 1 diabetes (29). Thus people with type 2 diabetes progressively resemble those with type 1 diabetes with respect to risk of hypoglycaemia.

Hypoglycaemia is a side-effect of sulphonylurea therapy. A study of sulphonylurea-induced hypoglycaemia that was of sufficient severity to require hospital treatment recorded an annual incidence of 4.2 per 1000 patients (43), but other European studies have estimated the frequency of severe hypoglycaemia to be much lower at 0.19-0.25 per 1000 patient years (44,45). A 2-year prospective trial that involved 321 subjects with type 2 diabetes, receiving treatment with either chlorpropramide or

glibenclamide, recorded an incidence of symptomatic hypoglycaemia of 19 episodes per 1000 patient years (46). Many studies have recorded higher rates of hypoglycaemia with long-acting sulphonylureas. A 12-year study in Switzerland demonstrated that treatment with long-acting sulphonylureas was three times more likely to precipitate admission to hospital with severe hypoglycaemia than the use of short-acting sulphonylureas (24). However, not all long-acting sulphonylureas provoke hypoglycaemia. The incidence of hypoglycaemia was reported to be low with the use of glimepiride which is administered once daily and stimulates insulin production primarily in response to meals (47). Almost all sulphonylurea drugs are metabolised in the liver to metabolites that are subsequently excreted in the urine. While most of these metabolites either have minimal or no metabolic activity, the two major hepatic metabolites of chlorpropamide do possess hypoglycaemic activity. Renal impairment prolongs the biological activity of many sulphonylureas and treatment should then be with a sulphonylurea that is metabolised mainly in the liver, such as gliclazide.

Table 1.5: Annual percentage (95% CI) of patients reporting at least one hypoglycemic episode in relation to demographic characteristics and therapy. Data derived from the UKPDS, 1998 (5).

	N	Any	Severe
Diet alone	756	0.8 (0.6 to 1.0)	0.1 (0.1 to 0.2)
Sulphonylurea	1418	7.0 (5.1 to 11.9)	1.2 (0.4 to 3.4)
Metformin	290	1.7 (1.0 to 3.0)	0.3 (0.1 to 1.1)
Basal insulin alone	1036	21.2 (14.6 to 29.8)	3.8 (1.2 to 11.1)
Basal plus prandial insulin	38	32.6 (21.8 to 45.6)	5.5 (2.0 to 14.0)

MORBIDITY AND MORTALITY

Effects on the Cardiovascular System (Table 1.6)

Acute hypoglycaemia provokes pronounced haemodynamic changes that include an increase in heart rate, stroke volume, myocardial contractility and cardiac output, and widening of pulse pressure (48,49). Regional changes in circulation occur during hypoglycaemia to increase the blood flow to vital organs such as the brain, and to the liver and skeletal muscle for the delivery of gluconeogenic precursors, while perfusion of non-essential organs such as the kidneys and spleen is reduced (49). Acute hypoglycaemia also has significant transient effects on peripheral blood, which encourage haemostasis and increase blood viscosity (49). These changes influence capillary blood flow and in some tissues may predispose to intravascular stasis during hypoglycaemia. The haemodynamic and haematological changes that occur during acute hypoglycaemia do not appear to compromise vascular perfusion in the normal healthy vasculature, but they may have potentially adverse effects in people with diabetes, in whom endothelial dysfunction and vascular disease are common, and in whom premature atherosclerosis and macrovascular disease are often present.

Hypoglycaemia may precipitate an acute vascular event or a dysrhythmia (49,50) in people who have pre-existing coronary heart disease or may compromise myocardial contractility in people who have the specific heart disease of diabetes. The secretion of adrenaline causes a rapid fall in plasma potassium (51), which could promote prolongation of ventricular repolarisation. Electrocardiographic changes, including prolongation of the QT interval, T wave flattening and inversion, and ST segment

depression, have been demonstrated during experimentally-induced hypoglycaemia (52), and transient cardiac arrhythmias have occasionally been demonstrated in non-diabetic and diabetic subjects during hypoglycaemia (50). These electrophysiological changes may lead to the generation of a serious cardiac arrhythmia, especially in the presence of occult coronary heart disease. Angina, myocardial infarction, acute cardiac failure and cardiac arrest have been described, albeit infrequently and anecdotally, as the direct consequences of acute hypoglycaemia (50,53). The true incidence of these events is unknown but may be underestimated in clinical practice because the precipitating factor of acute hypoglycaemia is either overlooked, may already have been treated, or counterregulation may have raised the blood glucose by the time the patient receives medical attention.

Table 1.6: Cardiovascular effects of acute hypoglycaemia

Physiological changes	Potential pathological sequelae
<ul style="list-style-type: none">• Increased heart rate• Widening of pulse pressure• Increased cardiac output• Electrophysiological changes (QT lengthening)	<ul style="list-style-type: none">• Silent myocardial ischaemia• Angina; myocardial infarction• Arrhythmias• Acute congestive cardiac failure (pulmonary oedema)

Physical injury

Physical injury occurring as a result of severe hypoglycaemia is relatively common. In a retrospective survey of people with insulin-treated diabetes (2), approximately 10% had suffered a physical injury related to severe hypoglycaemia at some time during their treatment with insulin. These included skeletal injuries with fractures and joint dislocations, head injuries and burns in addition to the more frequent minor soft tissue injuries of bruises, cuts and lacerations. Injuries may occur as a result of falls following dizziness, inco-ordination, drowsiness, loss of consciousness, or convulsions (54) and may be a consequence of driving-related accidents associated with hypoglycaemia.

Effects on the Central Nervous System

The effects of hypoglycaemia on the central nervous system are discussed in Chapter 1.2.

Potential effect on microvascular disease

The profound haemodynamic changes that occur in response to acute hypoglycaemia may have functional effects on vascular flow and may damage capillaries that are already abnormal. The sudden changes in arterial pressure and capillary blood flow may expose the microvasculature to fluctuating stress of varying magnitude. It has been postulated that these changes, in combination with increased plasma viscosity and coagulability, may affect the capillary circulation, reducing capillary blood flow, encouraging stasis, thrombosis and capillary closure and so

producing localised ischaemia and hypoxia in vulnerable tissues such as the retina and renal glomeruli (55). This may lead to capillary closure in tissues already affected by diabetic microvascular disease.

Anecdotal reports have associated nocturnal hypoglycaemia with acute vitreous haemorrhage in patients who had untreated proliferative diabetic retinopathy (56,57). The cause may be related to the sudden fall in intra-ocular pressure which occurs in response to acute hypoglycaemia (58), with the effects of sudden mechanical stress and changes in perfusion pressure causing rupture of delicate new vessels in the retina and vitreous humour. However, rapid improvement in glycaemic control in people with longstanding type 1 diabetes who have established background diabetic retinopathy is associated with a worsening of retinopathy (59). While the principal cause for the development of retinal ischaemia during strict glycaemic control is presumed to be the rapid reduction in the retinal hyperperfusion that is associated with chronic hyperglycaemia, it is tempting to speculate that recurrent acute hypoglycaemia may have a contributory role. Hypoglycaemia also causes an acute reduction in renal plasma flow and glomerular filtration (60), and theoretically may influence progression of glomerular and arteriolar sclerosis in patients with diabetic nephropathy, although at present there is no evidence to support this premise.

Mortality

Severe hypoglycaemia has been implicated in 2-4 % of deaths in the insulin-treated diabetic population (61,62). The number is difficult to estimate with accuracy because of the difficulty of identifying and confirming that death has directly resulted

from hypoglycaemia (61), particularly in the fatal outcome of an acute vascular event. The risk of sudden death in people with insulin-treated diabetes is higher than that of a non-diabetic population of equivalent age, and in younger people this is principally attributable to metabolic causes, of which severe hypoglycaemia predominates (62).

Hypoglycaemia has been implicated in the 'dead in bed' syndrome (63-65), in which young people with type 1 diabetes, who have been in good health and have been sleeping alone, are found dead in an undisturbed bed. The 'dead in bed' syndrome accounts for 6% of deaths amongst individuals with type 1 diabetes under 40 years of age, occurring at a rate of two to six events per 100,000 patient years (65). Hypoglycaemia-induced cardiac arrhythmia, acute respiratory arrest with impaired baroreflex sensitivity (66) or undetected autonomic dysfunction (67), have been suggested as possible causative mechanisms. The sudden development of a fatal cardiac arrhythmia is the most likely explanation as death following neuroglycopenic cerebral damage is relatively slow and produces characteristic histopathological changes in the brain.

PSYCHO-SOCIAL EFFECTS OF HYPOGLYCAEMIA

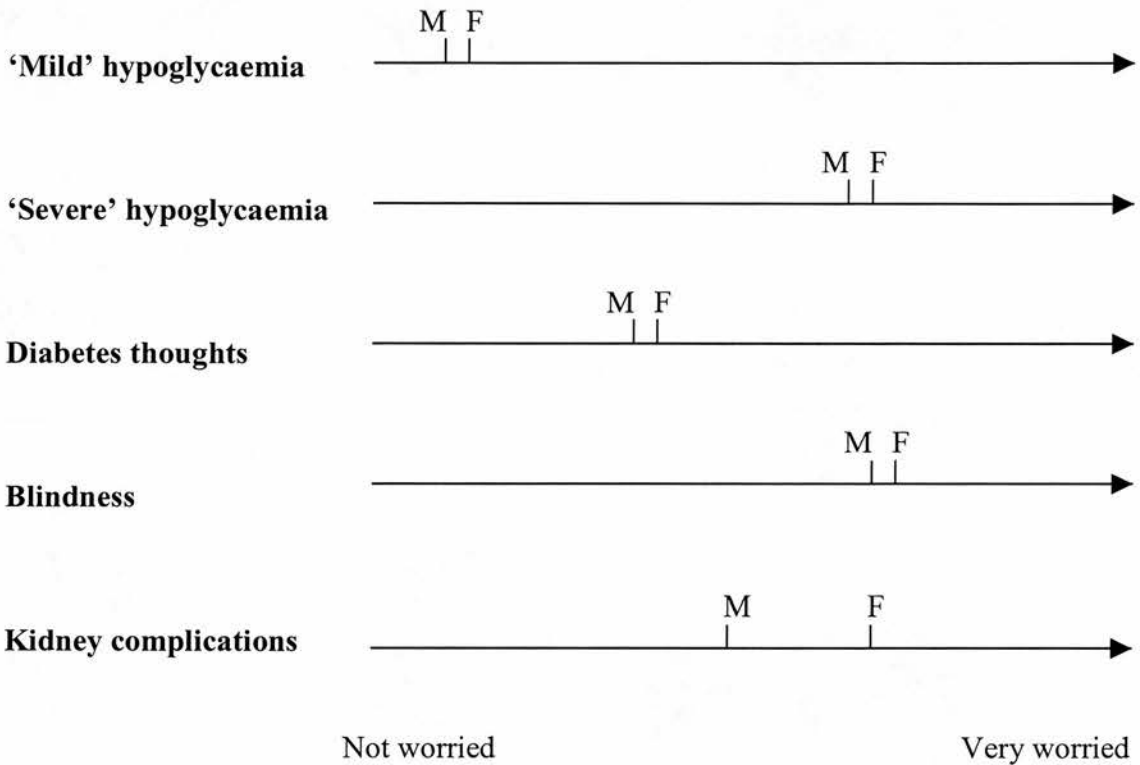
The manifestations of hypoglycaemia are unpleasant and frightening (68) and the consequences of severe hypoglycaemia can be serious. Severe hypoglycaemia is very disruptive and can impinge on every aspect of daily life. It can occur at any time of the day or night and is frequently unpredictable. The ever-present risk of hypoglycaemia and its possible ramifications often causes long-term emotional distress. Psychological



manifestations include decreased happiness, chronic anxiety and depression (69,70), and rarely may induce a phobia (71). Such is the fear of hypoglycaemia and its potential outcomes, that many people with diabetes regard it with the same degree of worry as the development of serious long-term complications of diabetes, such as sight-threatening retinopathy or renal failure (33) (Figure 1.2). Hypoglycaemia may also cause significant psychological upset in family members, particularly spouses or partners (72) and the parents of diabetic children (73). This can influence the approach to self-care of diabetes by the individual or by carers and relatives and may encourage poor or suboptimal glycaemic control in an attempt to avoid severe hypoglycaemia (74).

Hypoglycaemia is a potential hazard when driving (75,76), and can affect employment prospects. In most Western countries, vocational driving licences (for large goods vehicles and passenger carrying vehicles) are not permitted for drivers with diabetes taking insulin, and the development of impaired awareness of hypoglycaemia or recurrent severe hypoglycaemia can cause the revocation of an ordinary driving licence. Because hypoglycaemia is a potential risk in the workplace, in Europe and North America insulin-treated diabetic patients are excluded from dangerous or high-risk occupations, and occasionally may suffer discrimination by employers. The risk and development of hypoglycaemia at work can result in enforced changes in, and sometimes loss, of employment. In the longer-term, all of these problems may enforce lifestyle changes.

Figure 1.2: Results on a visual analogue scale, showing attitudes and concerns towards different aspects of diabetes, as indicated by patients with Type 1 diabetes. Reproduced from Pramming *et al*, 1991 (33), with the permission of Diabetic Medicine.



TREATMENT

Insulin-treated patients who are conscious and able to swallow should be given oral carbohydrate. Guidelines exist for the self-treatment of mild symptomatic hypoglycaemia, and suggest the initial consumption of 10–15 grams of fast-acting carbohydrate, preferably as glucose (e.g. 2-3 dextrose tablets) followed by longer-acting carbohydrate in the form of starch. Unconscious patients should receive 50% dextrose as an intravenous bolus of 20-50 ml and, if hypoglycaemia is protracted, an intravenous infusion of 10-20% dextrose may be required to maintain euglycaemia. Intramuscular or intravenous glucagon may also be given, but if the hypoglycaemia has been precipitated by, or is associated with, excessive alcohol intake, glucagon may be ineffective as alcohol blocks the glycogenolytic action of glucagon to convert hepatic glycogen into glucose. Glucagon is not recommended for the treatment of sulphonylurea-induced hypoglycaemia on the theoretical basis that it can stimulate further endogenous insulin release. Patients who are being treated with sulphonylureas may need admission to hospital for the treatment of severe hypoglycaemia. A long-acting sulphonylurea such as glibenclamide has a long half-life and the hypoglycaemia may therefore persist or recur, necessitating maintenance of a dextrose infusion for 3 to 5 days.

PREVENTION

Prevention is better than cure and it is essential that newly diagnosed patients with type 1 diabetes, and people with type 2 diabetes who are commenced on insulin therapy, receive adequate education about the potential risks of hypoglycaemia, its treatment and prevention. Instruction on the prevention and emergency treatment of hypoglycaemia should embrace the patient's relatives and friends, and if in employment, the employer and colleagues at work should also be informed. Because the symptoms of hypoglycaemia are idiosyncratic, early familiarisation of patients with the symptoms that are peculiar to themselves is important. Because the nature and intensity of symptoms may alter with time, the symptoms of hypoglycaemia should be reviewed at regular intervals (14). The relationships between insulin administration and timing of meals must be explained, and specific advice is required for driving, premeditated exercise, including sport, and holidays. Specific subgroups, such as people who have impaired awareness of hypoglycaemia (Table 1.7), and those who have a history of recurrent nocturnal hypoglycaemia (Table 1.8) may require additional advice. There is some evidence that some individuals who have developed impaired awareness of hypoglycaemia may regain their symptomatic awareness if hypoglycaemia can be strictly avoided (77-80), although this is often very difficult to achieve in clinical practice. However, it may be possible with the utilisation of newer treatments and technology such as a wider range of insulin analogues, insulin pumps (continuous subcutaneous insulin infusion) and the imminent prospect of methods of continuous blood glucose monitoring.

Short-acting insulin analogues, which have a rapid onset and short duration of action, are effective in reducing the incidence of hypoglycaemia in people with type 1 diabetes (81). They are of particular benefit in people with unpredictable lifestyles, as they allow greater flexibility in timing and dosage of insulin and timing of meals. The long-acting insulin analogue, insulin glargine, has been shown in some studies to be beneficial in reducing the incidence of hypoglycaemia, particularly at night (81).

Other important prophylactic measures include the choice of appropriate insulin regimens and dose schedules, or anti-diabetic drugs in type 2 diabetes, and the establishment of appropriate therapeutic goals. Intensive insulin therapy is contraindicated in people with impaired awareness of hypoglycaemia and in those with a history of recurrent severe hypoglycaemia, because of the risks of strict glycaemic control in these conditions. For people with type 2 diabetes, precautions need to be undertaken to avoid hypoglycaemia associated with sulphonylurea therapy (Table 1.9).

Table 1.7: Treatment strategies for people with impaired awareness of hypoglycaemia

- Frequent blood glucose monitoring
- Avoid blood glucose values < 4.0 mmol/l
- Avoid maintenance of HbA1c within non-diabetic range
- Use predominantly short-acting insulins (e.g. basal-bolus regimen; use of insulin analogues)
- Consume regular snacks between meals and at bedtime

Table 1.8: Measures to avoid nocturnal hypoglycaemia

- Measure blood glucose at bedtime
- Bedtime snack containing long-acting carbohydrate
- Do occasional blood glucose measurements during the night
- Take care with timing of evening meals and timing of insulin administration
- Defer administration of intermediate-acting insulin until bedtime
- Use rapid-acting insulin analogue before evening meal
- Use long-acting insulin analogue (taken in morning)
- Take care with consumption of alcohol and strenuous exercise during evening
- Keep rapid acting carbohydrate at bedside for emergency treatment

Table 1.9: Avoidance of hypoglycaemia associated with anti-diabetic drugs

- Use short-acting drugs (e.g. gliclazide, glipizide) or long-acting derivative glimepiride (lower risk of hypoglycaemia)
- Check renal, hepatic and cardiac function
- Consider discontinuing anti-diabetic drugs if HbA1c is within the non-diabetic range
- Reduce dose or discontinue antidiabetic drugs if food intake is substantially reduced (e.g. intercurrent illness)

Chapter 2

Effects of Hypoglycaemia on the Brain

In humans the most important organ to be affected by acute hypoglycaemia is the brain.

CEREBRAL EVENTS

Convulsions

Electrophysiological manifestations of cerebral dysfunction accompany the cognitive and mood changes associated with acute hypoglycaemia. Characteristic electroencephalographic (EEG) changes occur during acute hypoglycaemia in the waking state. A decrease in alpha waves is accompanied by an increase in delta and theta waves, which are more pronounced over anterior parts of the brain (82,83). These changes are more prominent in people with type 1 diabetes in whom EEG evidence of epileptiform activity during hypoglycaemia has been recorded more commonly than in non-diabetic subjects (84). Acute neuroglycopenia is a recognised cause of focal or generalised convulsions, and in an individual treated with insulin it is important to exclude acute hypoglycaemia as the cause of a seizure. EEG changes often persist for several days after a hypoglycaemia-induced convulsion, so neurophysiological investigations should be deferred for at least a week, and blood glucose should be estimated at the time of performing an EEG. Permanent EEG changes are present in many people who have a history of recurrent severe hypoglycaemia (82).

Cerebrovascular Events

Transient hemiplegia is another alarming manifestation of acute hypoglycaemia (85), although it is unclear whether the mechanism is the direct effect

of neuroglycopenia or whether altered regional blood flow causes localised ischaemia within the brain. In older persons, acute changes in regional cerebrovascular perfusion may precipitate an acute vascular event such as a transient ischaemic attack or even a stroke.

Coma

Coma is relatively uncommon and constitutes about one third of all episodes of severe hypoglycaemia. Fortunately, prolonged neuroglycopenia associated with hypoglycaemic coma is rare but, when it is associated with the development of cerebral oedema, the mortality is high. Many of these fatal cases are associated with a deliberate overdose of insulin in an individual with suicidal intent (86) or are associated with the ingestion of an excessive quantity of alcohol (87). The few individuals who survive such protracted, profound neuroglycopenia often have extensive and permanent neurological damage, associated with severe cognitive impairment and memory loss, and they may remain in a chronic vegetative state (88). In cases with such a disastrous outcome, neuroimaging usually demonstrates cortical and hippocampal atrophy with secondary ventricular enlargement.

Mechanisms of hypoglycaemia-induced brain injury may involve the release of toxic neuro-excitatory amino acids, such as glutamate and aspartate (89,90), and localised cerebral ischaemia. In humans, the duration of severe hypoglycaemia is probably more important than the depth in causing damage to the brain. Neuropathological observations have confirmed the rostrocaudal direction of vulnerability of the brain to neuroglycopenia. The cerebral cortex and hippocampus

are most sensitive while the hindbrain and spinal cord are relatively resistant to neuroglycopenia (91,92).

EFFECTS OF HYPOGLYCAEMIA ON COGNITIVE FUNCTION

Acute

The human brain is the most important organ to be affected by acute hypoglycaemia, because of its dependence on a continuous supply of glucose. Progressive neuroglycopenia causes cognitive impairment (93,94), manifested initially as reduced speed of cerebration and difficulty concentrating, but proceeding to drowsiness, confusion, and ultimately loss of consciousness. Complex tasks that require rapid decision-making, sustained attention and planning capabilities deteriorate during mild to moderate hypoglycaemia, whereas simple cognitive and motor tasks are more resistant and may be relatively unaffected (94). Considerable inter-subject variability exists in the neuropsychological responses of individuals to hypoglycaemia. Some people may develop severe cognitive impairment during hypoglycaemia, while others are relatively unaffected at a similar blood glucose concentration. Individual differences in susceptibility to the cognitive effects of acute hypoglycaemia are mediated by a large number of interacting factors, some of which have been determined (94-99). These include male gender, high IQ and having type 1 diabetes, with or without impaired awareness of hypoglycaemia. Mood changes are also common during acute hypoglycaemia and include tense-tiredness, anger, depression, anxiety and pessimism. Denial that hypoglycaemia is present is common, and irrational and aggressive behaviour may sometimes occur. Occasionally a state

of automatism is said to develop, although there have been no scientific studies of this phenomenon. Recovery of cognitive function is usually fairly rapid after the blood glucose concentration returns to normal with most aspects of intellectual function returning to normal within 45-90 minutes (100,101). Even after severe hypoglycaemic coma, recovery of cognition is complete within 24 hours (101). However, disturbance of mood and memory and a feeling of general malaise may persist for longer (102), and amnesia of severe hypoglycaemia may occur.

Chronic

In recent years, evidence has accumulated to suggest that diabetes per se may be associated with the gradual emergence of modest cognitive impairment (103-106). This presumably develops as a result of prolonged exposure to hyperglycaemia and other metabolic abnormalities, and is compounded by the effects of hypertension and vascular disease on the brain. Potential causes of cognitive impairment in diabetes are listed in Table 2.1. Among these, repeated exposure to severe hypoglycaemia has been postulated to cause progressive cognitive dysfunction in some people with type 1 diabetes. This premise is controversial and is therefore reviewed in some detail.

Occasional non-diabetic patients who have been subjected to chronic neuroglycopenia as a result of an insulinoma have been reported to develop significant and permanent deterioration in intellectual function (107). Anecdotal case reports in people with insulin-treated diabetes have suggested that recurrent exposure to severe hypoglycaemia may have lasting consequences, resulting in a chronic deterioration of cognitive function with reduced intellectual ability, impaired social

skills and behaviour, inability to maintain employment and increasing dependence on a spouse or carer (69). The age of onset of diabetes is an important determinant of the potential effects that recurrent hypoglycaemia may have on cognitive function and intellectual ability, with children being the most susceptible.

Table 2.1: Putative causes of cognitive impairment in diabetes.

Chronic hyperglycaemia
Recurrent severe hypoglycaemia
Cerebrovascular disease; ischaemia, infarction
Recurrent diabetic ketoacidosis
Depression; psychiatric disorders
Hypertension
Psychosocial impact of chronic disease
Alcohol, drug abuse

Effects of recurrent hypoglycaemia in childhood

The developing and immature brain of the young child is particularly vulnerable to the effects of neuroglycopenia. The deleterious influence of hypoglycaemia on intellect and cerebral function has been shown to be greatest in children who have developed diabetes before the age of five years and who have been exposed to multiple episodes of severe hypoglycaemia in early childhood, especially when these have provoked convulsions (108-110). Cognitive dysfunction may become manifest later in childhood or adolescence with demonstrable learning difficulties, and occasionally with abnormal behaviour (111).

Several retrospective, cross-sectional (108,109), and prospective studies (111,112) have shown an association between the early onset of type 1 diabetes in childhood and lower than expected powers of attention and psychomotor efficiency in adolescence, when compared to non-diabetic peer groups (113). A direct correlation has been demonstrated between the frequency of hypoglycaemia-induced convulsions at this age and evidence of cognitive dysfunction later in life (110,114). Children with onset of diabetes *after* the age of five years also perform less well than their non-diabetic peer group on many tests of cognitive function, especially on assessment of verbal IQ. However, this is considered to be a consequence of the psychosocial and educational effects of diabetes, with factors such as interrupted school attendance through ill-health or intermittent metabolic disturbances playing a significant role, rather than a direct pathophysiological effect of repetitive hypoglycaemia on the brain (110).

The relationship between cognitive dysfunction and the frequency of exposure to severe hypoglycaemia has been examined in adults with insulin-treated diabetes but with less definitive results than those observed in children. Two major prospective studies, the DCCT (115) and the SDIS (36), have not shown an association between the frequency of severe hypoglycaemia and measures of cognitive function (116). Both of these studies were designed to evaluate the effect of strict glycaemic control on limiting the complications of diabetes. The participants were subdivided into intensively-treated and conventionally-treated subgroups, achieving either strict or moderate glycaemic control respectively; the incidence and progression of diabetic complications and the frequency of adverse effects of treatment were monitored prospectively. Both studies have shown unequivocally that strict glycaemic control limits the development and progression of diabetic microangiopathy, but is accompanied by a higher rate of severe hypoglycaemia.

In the DCCT, a comprehensive battery of tests of cognitive function was applied, whereas the cognitive tests used in the SDIS were much more limited, and a clear separation of the groups was not achieved in terms of exposure to severe hypoglycaemia (36). However, in both studies the higher frequency of severe hypoglycaemia in the patients with strict glycaemic control was not associated with any evidence of intellectual decline.

While these results are reassuring and suggest that mental ability is not being affected adversely by repeated exposure to severe hypoglycaemia, they need to be interpreted with caution. Both the DCCT and SDIS had stringent recruitment criteria. The participants, and the extent of medical support available to them, were not

representative of the insulin-treated diabetic population in general. They were relatively young and had diabetes of short duration. They were above average intelligence, were highly motivated and were well educated in self-care of their diabetes. People who had a history of multiple episodes of severe hypoglycaemia were excluded. This criterion is likely to have excluded anyone with impaired hypoglycaemia awareness, so that few, if any, patients were recruited to participate in these studies who were at high risk of developing severe hypoglycaemia. It would be imprudent, therefore, to extrapolate the conclusions from these studies to the archetypal population with type 1 diabetes which is much more heterogeneous, and includes people who have impaired awareness of hypoglycaemia and other risk factors for severe hypoglycaemia. The rate of severe hypoglycaemia in unselected populations is double that observed in the DCCT group who had strict glycaemic control (1,33). Furthermore, cognitive decline may be a late manifestation of the cumulative effects of severe hypoglycaemia, emerging after 20 years or more of treatment with insulin, and the causes may be multifactorial. The timescale of the follow-up (less than 10 years) of these two major prospective studies may not have been long enough to reveal the development of an intellectual deficit.

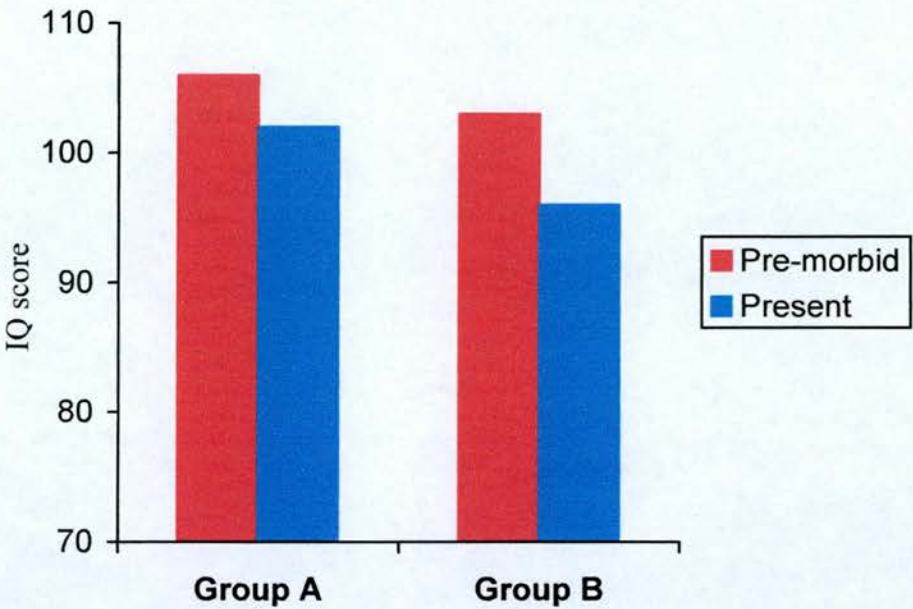
By contrast, studies in adult patients with type 1 diabetes which have relied on a retrospective estimate of the frequency of exposure to recurrent severe hypoglycaemia have demonstrated a significant correlation with evidence of impaired intellectual function. In an early study, measures of global intelligence in 100 adults with insulin-treated diabetes were compared with demographically matched, non-diabetic controls (117). Intellectual function was impaired in the diabetic group compared to the control group and the cognitive decrement appeared

to be related to the estimated number and severity of previous episodes of hypoglycaemia (117). An association between the estimated number of previous severe hypoglycaemic events and the neuropsychological test performance of adults with type 1 diabetes has been observed in other studies (118,119). In a Swedish study of a small group of 17 adults with type 1 diabetes all of whom had a history of recurrent severe hypoglycaemia, deficits were demonstrated in tests of problem solving, motor ability, visuospatial skills and in tasks which assess frontal lobe functions (119). However, the study design did not preclude the possibility that the patients who had a history of recurrent hypoglycaemia may have been those who had a lower pre-morbid IQ and, being less adept in their self-management of diabetes, had therefore experienced a higher frequency of severe hypoglycaemia.

This possibility was avoided in a study in Edinburgh (120), in which the degree of intellectual impairment was estimated as the difference between measures of premorbid and current IQ. A significant correlation was demonstrated between the degree of cognitive impairment and the frequency of severe hypoglycaemia in a group of 100 adults who had developed type 1 diabetes in adulthood (i.e. after full maturation of the brain and attainment of intellectual ability). Partial correlation analysis showed that the observed correlations were not related to age, duration of diabetes or blood glucose measured at the time of cognitive testing. The group could be divided approximately into quartiles on the basis of their previous experience of severe hypoglycaemia. These subgroups were matched for age, duration of diabetes, education, social class and premorbid IQ. Comparison of the quartile who had a history of five or more episodes of severe hypoglycaemia with the quartile with no previous severe hypoglycaemia, revealed significant differences in IQ deficit (Figure

2.1), performance IQ and reaction time (121). Individual differences in the experience of severe hypoglycaemia were not related to differences in premorbid intelligence.

Figure 2.1: Comparison of premorbid (red bars) and present (blue bars) IQ levels between a group of individuals with Type 1 diabetes with no history of previous severe hypoglycaemia (group A) and a group of individuals with Type 1 diabetes with a history of five or more previous episodes of severe hypoglycaemia (group B). Premorbid versus present IQ comparison for group A is not significant, for group B it is significant at $p < 0.001$. Reproduced from Langan *et al*, 1991 (120), with the permission of Diabetologia.



While this study indicated a relationship between the frequency of severe hypoglycaemia and cognitive impairment (119,120), it could not exclude the possibility that diabetes *per se* was influencing cognitive function. The subjects with type 1 diabetes were therefore compared with a group of non-diabetic controls, matched for pre-morbid IQ, age and educational experience. This demonstrated that the performance and verbal IQs of the diabetic participants were lower than the non-diabetic controls (121). Within the group of subjects with diabetes, the effect of previous severe hypoglycaemia was associated specifically with a deficit in performance IQ, and decision-making and response initiation skills were affected adversely in preference to processes that involved the encoding and storage of information (122). The lower verbal IQ in the subjects with type 1 diabetes may therefore be the consequence of other factors, such as the social and educational impact of having this disorder. A different study which used an identical protocol and selection criteria for a group of 70 subjects with type 1 diabetes, was performed subsequently in Nottingham and reported very similar findings (123)

These studies have shown the existence of a positive correlation between the frequency of severe hypoglycaemia and a modest degree of intellectual impairment. Although the mean decrement in intellectual ability resulting from recurrent severe hypoglycaemia in the Edinburgh study was small (approximately 6 IQ points), this deficit is significant (equivalent to half a standard deviation) and potentially important (120-122). It would be sufficient to influence competence in tasks that require intellectual dexterity and could adversely affect individuals who have intellectually demanding occupations, in terms of job performance and capability. While this mean decrement represented the *average* effect of recurrent severe

hypoglycaemia on performance IQ in the group, some individuals were affected to a greater degree. It is also pertinent to emphasise that the participants had developed type 1 diabetes in adulthood, after full intellectual ability had been achieved, and that very strict inclusion criteria were applied to avoid confounding variables that could have affected cognitive function adversely, such as hypertension, previous head injury, psychiatric illness and alcohol abuse. In most people with type 1 diabetes, the modest cognitive deficits indicated by the retrospective studies are unlikely to be obvious in the performance of everyday tasks as the decline in intellectual function is small and appears to be slowly progressive. In older people intellectual decline may be attributed to the ageing process and disregarded.

While these retrospective studies suggest that recurrent exposure to severe hypoglycaemia may cause a modest decline in cognitive function, a major criticism is that they are limited by the accuracy of recall of previous hypoglycaemic events. However, in some studies (120,121,123) the accuracy of individual reporting was enhanced by interviewing relatives and by scrutiny of medical records. In addition, the cohort with type 1 diabetes in the original Edinburgh study (120) was reassessed within a period of 18 months, and their estimated frequency of severe hypoglycaemia showed good reliability with the rate that had been reported previously (122), so helping to validate the method used to estimate the frequency of previous severe hypoglycaemia.

A further piece of supportive evidence comes from clinical and experimental observations of people with type 1 diabetes who have chronic impairment of awareness of hypoglycaemia (see later section) who have a high frequency of severe hypoglycaemia. In these individuals cognitive dysfunction is more profound during

acute hypoglycaemia (124,125), and persists for longer following restoration of normoglycaemia than in unaffected people with type 1 diabetes who have normal awareness of hypoglycaemia. In a population study, a modest decline in intellectual function was noted to be associated with impaired awareness of hypoglycaemia (126). In addition, a study of the speed of recovery of cognitive function following a single episode of severe hypoglycaemic coma in a group of people with type 1 diabetes, suggested that cognitive decrements and altered mood states in those with a history of recurrent severe hypoglycaemia were persistent and were probably a consequence of previous repeated exposure to severe neuroglycopenia (101). This suggests that the frequent exposure to severe hypoglycaemia suffered by people with type 1 diabetes who have impaired awareness of hypoglycaemia may have a long-term detrimental effect on intellectual function.

In conclusion, it is possible that repeated episodes of severe hypoglycaemia may have a deleterious effect on cognitive function that is cumulative over time and is akin to the chronic intellectual impairment associated with repeated exposure to other acute neurotoxic insults, such as alcohol abuse, hypoxia (sleep apnoea) and repetitive head injury (boxing). Individuals may vary in their susceptibility to hypoglycaemic injury, and a wide spectrum of abnormality is likely, with only a few individuals developing severe intellectual impairment (69). However, although the results of studies are suggestive that recurrent hypoglycaemia in adults may have a deleterious long-term effect on cognitive function, the evidence is still equivocal and remains unproven.

STRUCTURAL CHANGES IN THE BRAIN IN DIABETES: ROLE OF HYPOGLYCAEMIA

If cognitive impairment is a permanent consequence of recurrent severe hypoglycaemia, could this cause structural changes in the brain in people with diabetes? Most neuroimaging studies of the brain have been performed in people with type 2 diabetes and have shown that structural degenerative changes are more common than in non-diabetic controls of similar age (127). The clinical significance of these neuroimaging abnormalities is not yet known, but is suggestive of a premature ageing of the brain. Cortical atrophy is present in 36-53% of subjects with type 2 diabetes compared to 12% of matched non-diabetic controls, occurs earlier in life and is more extensive in people with diabetes (127). Leukoaraiosis, a patchy area of either focal or diffuse demyelination and gliosis, is a non-specific, age-related finding and is associated with several pathological conditions, including hypertension, vascular disease, demyelination and dementia (128). It has been identified in 69% of subjects with diabetes compared to 12% of non-diabetic controls of similar age (129). A significantly higher incidence of degenerative changes in the brain has also been reported in subjects with type 1 diabetes compared to age-matched non-diabetic controls (130,131) but the cause is unknown.

A few studies have tried to identify whether structural changes in the brains of diabetic subjects are associated with deficits in intellectual performance (132-134) and to date the results have been inconclusive. Magnetic Resonance Imaging (MRI) scans of the brain were performed in a subgroup of subjects who had participated in the major Edinburgh study described earlier (120-122) and an association was observed between the presence of cortical atrophy and a history of recurrent severe

hypoglycaemia (131). However, the number of people in this study was too small to demonstrate any significant correlation with impaired cognitive function. Furthermore, it is possible that type 1 diabetes *per se* could be an important pathogenetic factor. This premise has been supported by the preliminary results of another large study in our centre of young patients who had developed type 1 diabetes in childhood or adolescence, and in whom any abnormalities demonstrated by neuroimaging (using MRI) were related to an assessment of their cognitive function (135). No correlation has been observed between structural changes in the brain and any abnormality of cognitive function, but MRI changes were more common in a subgroup of patients who had diabetic retinopathy. This would suggest that exposure to chronic *hyperglycaemia* may be of greater importance than hypoglycaemia in causing cerebral changes in young people with type 1 diabetes. This interpretation is consistent with observations from recent studies by Wessels *et al* (136,137), and from the results of the Epidemiology of Diabetes Interventions and Complications (EDIC) study (138). Wessels *et al* (136,137) have shown that people with Type 1 diabetes, who, as a consequence of chronic hyperglycaemia, had developed advanced retinopathy, had also developed evidence of microvascular damage in the brain, and the results of the EDIC study, an 18-year follow-up of the DCCT cohort, have indicated that higher glycated HbA1c values, and not frequency of severe hypoglycaemia, were associated with psychomotor impairment (138). An earlier American study of young adults with type 1 diabetes (139), demonstrated that impaired cognitive function was associated with peripheral neuropathy (a surrogate marker of chronic hyperglycaemia) and not with previous severe hypoglycaemia *per se*. However, when the effects of peripheral neuropathy and severe hypoglycaemia

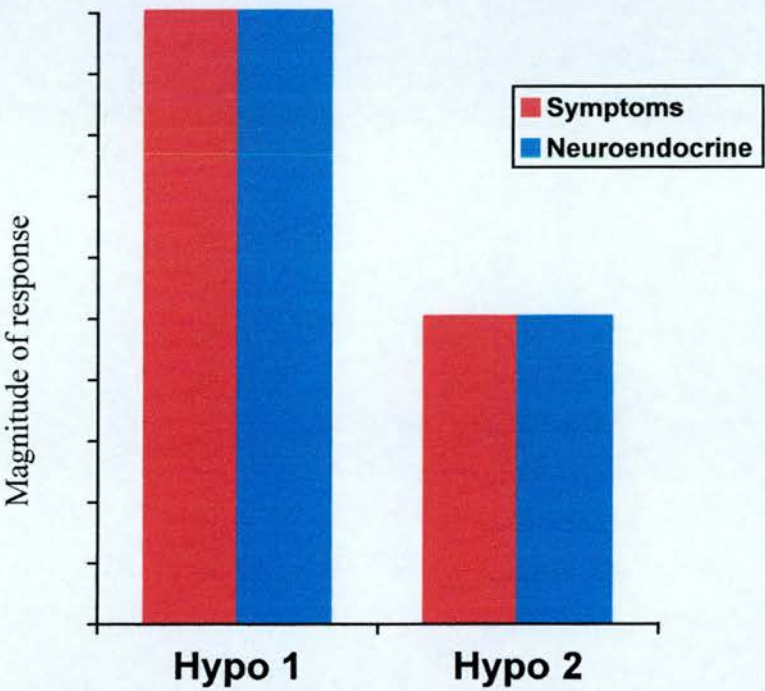
were combined, a significant correlation with cognitive dysfunction was observed suggesting that exposure to severe hypoglycaemia compounds the adverse effect of poor glycaemic control on cerebral function. Similar results have been reported in a recent study by Musen *et al* (140), who have demonstrated that both persistent hyperglycaemia and exposure to acute severe hypoglycaemia have an impact on brain structure.

It is likely that when cognitive dysfunction does develop in people with insulin-dependent diabetes, this has a multifactorial pathogenesis. Several metabolic factors, including chronic hyperglycaemia (possibly with intermittent exposure to diabetic ketoacidosis) and recurrent hypoglycaemia, may interact to affect neurological function. In addition, evidence is accumulating for the existence of a central neuropathy (or encephalopathy) in people with type 1 diabetes of long duration (103-106), which is presumed to have a multifactorial pathogenesis, and to which severe hypoglycaemia may contribute.

ACQUIRED HYPOGLYCAEMIA SYNDROMES

Hypoglycaemia is associated with the development of acquired clinical syndromes that are specific to insulin-treated diabetes, which may be the consequence of recurrent exposure to hypoglycaemia *per se*. A single protracted episode of hypoglycaemia lasting for more than an hour is associated with diminished magnitude of the symptomatic and neuroendocrine responses to a further episode of hypoglycaemia occurring within the ensuing 48 hours (141) (Figure 2.2). Recurrent exposure to hypoglycaemia, through the pathogenetic role of antecedent hypoglycaemia, may induce these acquired hypoglycaemia syndromes, in which the glycaemic thresholds for symptomatic and neuroendocrine responses are modified as a result of cerebral adaptation.

Figure 2.2: Schematic diagram to represent the effect of antecedent hypoglycaemia on symptomatic and neuroendocrine responses to subsequent episodes of hypoglycaemia. From *Hypoglycaemia in Clinical Diabetes*, 1999 (eds Frier BM, Fisher BM), with the permission of the publishers.



Impaired awareness of hypoglycaemia

Various mechanisms have been proposed to underlie the aetiology of impaired awareness of hypoglycaemia and it is possible that several co-existing factors are important in inducing this condition (Table 2.2). Recurrent hypoglycaemia appears to be of particular importance in the development of impaired awareness of hypoglycaemia (28). Although impaired awareness of hypoglycaemia is associated with strict glycaemic control in the short term, it may also be a long-term consequence of recurrent exposure to hypoglycaemia. People with type 1 diabetes of long duration who have developed impaired awareness of hypoglycaemia, irrespective of the quality of glycaemic control, have been shown to have an altered glycaemic threshold for autonomic symptoms, which is set at a lower than usual blood glucose concentration (27,28,142). Neuroglycopenia develops before autonomic symptoms are generated, thus interfering with subjective perception of warning symptoms and ability to self-treat the low blood glucose.

Table 2.2: Possible pathogenetic mechanisms of impaired awareness of hypoglycaemia. From *Hypoglycaemia in Clinical Diabetes*, 1999 (eds Frier BM, Fisher BM).

CNS adaptation

Chronic exposure to low blood glucose

- Strict glycaemic control in people with diabetes

Recurrent transient exposure to low blood glucose

- Antecedent hypoglycaemia

CNS glucoregulatory failure

- Counterregulatory deficiency
- Hypoglycaemia associated central autonomic failure

Peripheral nervous system dysfunction

- Peripheral autonomic neuropathy
- Reduced peripheral adrenoceptor sensitivity

Counterregulatory hormonal deficiencies

The magnitude of counterregulatory hormonal responses to hypoglycaemia often declines with increasing duration of diabetes. Hypoglycaemia-induced secretion of glucagon becomes deficient in most people within five years of onset of type 1 diabetes (143,144), and may be followed within a few years by attenuation of the adrenaline response to hypoglycaemia (144,145). People with type 1 diabetes who have counterregulatory deficiencies are at greater risk of progressing to severe hypoglycaemia if their blood glucose falls, particularly if they are subjected to intensive insulin therapy (146). Similar counterregulatory hormonal deficiencies have been demonstrated in people with insulin-treated type 2 diabetes who have progressed to pancreatic beta cell failure (147).

In some people with longstanding type 1 diabetes, peripheral autonomic neuropathy may contribute to a subnormal adrenaline secretory response. However, in people who have counterregulatory hormonal deficiencies, the blood glucose threshold at which the adrenaline secretory response to hypoglycaemia is triggered is altered, so that a lower blood glucose (i.e.. a more profound hypoglycaemic stimulus) is required to initiate secretion of adrenaline (148,149). This shift in glycaemic threshold is probably a consequence of cerebral adaptation to exposure to recurrent hypoglycaemia.

Cerebral adaptation: altered glycaemic thresholds

Cerebral adaptation can occur following short and longer term exposure to hypoglycaemia, which causes elevation of the glycaemic thresholds at which the secretion of counterregulatory hormones and the onset of symptoms of

hypoglycaemia occur (i.e. a lower blood glucose has to be reached and a more profound hypoglycaemic stimulus is therefore necessary) (150-152). The putative mechanism appears to be an ability of the brain to adapt to function normally despite chronic exposure to a low blood glucose concentration (153,154). Attenuation of the neuroendocrine and symptomatic responses occurs in type 1 diabetes when the glycated haemoglobin concentration is within or near to the non-diabetic range (155). Such patients can maintain normal glucose uptake by the brain during hypoglycaemia (154), so preserving cerebral metabolism and delaying the neuroendocrine and symptomatic responses to hypoglycaemia, and so blunting symptomatic awareness of hypoglycaemia. Although these changes initially protect the brain from a greater degree of neuroglycopenia, they are thought to be maladaptive responses, as the normal symptomatic warning is suppressed, narrowing the margin for corrective action to be taken and so risking the development of more severe neuroglycopenia. In people with well controlled type 2 diabetes (on diet or antidiabetic agents), the counterregulatory and symptomatic responses occurred at higher blood glucose concentrations than in type 1 diabetes and in non-diabetic controls (156,157), but this glycaemic threshold was altered when people with type 2 diabetes required treatment with insulin (158). The phenomenon of cerebral adaptation to strict glycaemic control in type 1 diabetes appears to be related to the use of insulin therapy, but is also associated with insulin deficiency. The glycaemic threshold for cognitive dysfunction is probably also susceptible to cerebral adaptation, but this remains a controversial issue. Some studies of people with type 1 diabetes have shown that despite strict glycaemic control or exposure to antecedent hypoglycaemia, cognitive function remains preserved during experimental

hypoglycaemia with a shift in glycaemic threshold for the onset of cognitive dysfunction (29,159-162). However, in other studies, cerebral adaptation to protect cognitive function did not appear to occur, and it became impaired at the same blood glucose threshold irrespective of preceding glycaemic experience (152,163-165). Such an outcome would allow cognitive dysfunction to occur at a higher plasma glucose concentration than that required to trigger the warning symptoms, thereby increasing the risk of severe neuroglycopenia and limiting the scope for intensive insulin therapy.

A preceding (antecedent) episode of hypoglycaemia can also promote cerebral adaptation, by altering the glycaemic thresholds for symptomatic and neuroendocrine responses to hypoglycaemia (166). Recurrent exposure to hypoglycaemia may therefore, through the pathogenetic role of antecedent hypoglycaemia causing cerebral adaptation, induce the acquired hypoglycaemia syndromes, with modification of the glycaemic thresholds for symptomatic and neuroendocrine responses.

Cerebral adaptation: altered regional blood flow

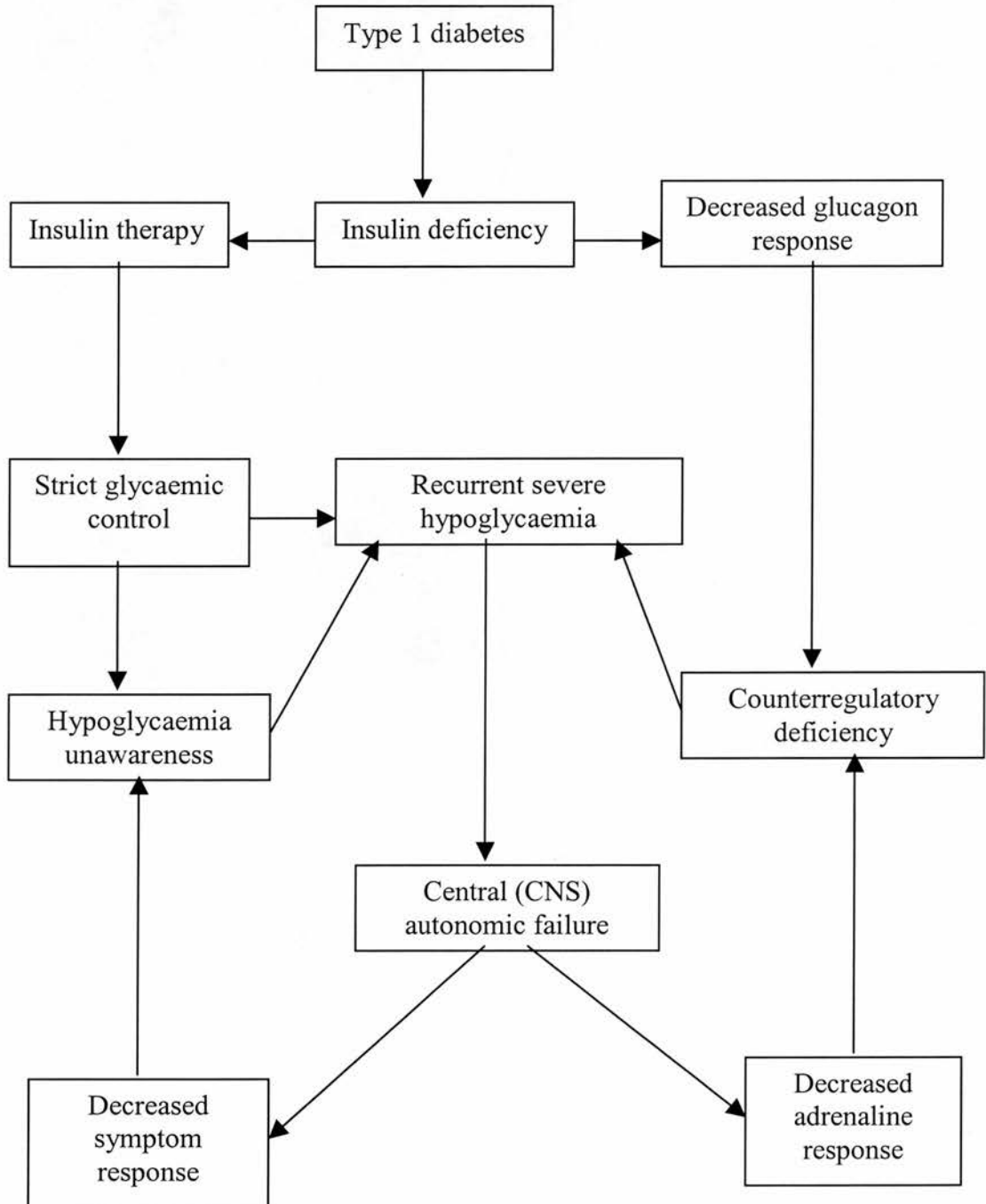
A permanent redistribution of regional cerebral blood flow occurs in people with type 1 diabetes who have a history of recurrent severe hypoglycaemia, with a relative but persistent increase in blood flow to the frontal lobes (167). This mirrors the normal physiological response to hypoglycaemia (168), and may represent a chronic adaptive response to protect the most vulnerable parts of the brain from the effects of further severe hypoglycaemia. However, it has been observed that the changes in regional cerebral blood flow in response to controlled hypoglycaemia

occur independently of the state of awareness of hypoglycaemia in people with type 1 diabetes (169), so this is presumably an adaptive response to hypoglycaemia *per se*.

Hypoglycaemia-associated autonomic failure

It has been suggested by Cryer that the acquired hypoglycaemia syndromes represent a form of 'hypoglycaemia-associated autonomic failure' (170) (Figure 2.3), implicating recurrent severe hypoglycaemia as the central problem that causes the development of these clinical syndromes. It is proposed that recurrent exposure to severe hypoglycaemia promotes cerebral adaptation, with resultant alteration of glycaemic thresholds, and the emergence of counterregulatory hormonal deficiencies and sympatho-adrenal insufficiency, culminating in a chronically impaired awareness of hypoglycaemia. Repetitive episodes of hypoglycaemia may lead to 'downregulation' of the central mechanisms that normally would respond to a low blood glucose to activate glucoregulatory responses and produce the warning symptoms of hypoglycaemia. As a result, a vicious circle is established so that hypoglycaemia promotes further episodes of severe hypoglycaemia, so perpetuating the problem.

Figure 2.3: Schematic diagram to demonstrate the model of hypoglycaemia-associated autonomic failure, derived from Cryer (1992), from *Hypoglycaemia in Clinical Diabetes*, 1999 (eds Frier BM, Fisher BM), with the permission of the publishers.



Chapter 3

Moderators of Cognitive Function During Acute

Hypoglycaemia in Humans

INTRODUCTION

Hypoglycaemia is a common side-effect of insulin treatment (1,33), which can impact upon all aspects of everyday life (171). Several important prospective studies have confirmed the long-term beneficial effects of strict glycaemic control, both in type 1 (3,4) and type 2 diabetes (172), so encouraging the use of intensive insulin therapy. However, this is associated with an increased risk of severe hypoglycaemia (3,4,173), which has recognised mortality and morbidity (2,50,174), and is feared by people with insulin-treated diabetes (171) and their relatives.

Acute hypoglycaemia induces a series of hormonal, symptomatic, neurophysiological and cognitive changes, which occur at different and defined blood glucose levels (glycaemic thresholds) (12). These thresholds are reproducible in non-diabetic individuals, but are dynamic in people with diabetes and can be altered by external factors such as very strict glycaemic control or recent preceding (antecedent) hypoglycaemia. The glycaemic thresholds at which the secretion of the counterregulatory hormones commences are just below the normal physiological range of blood glucose (arterial blood glucose concentration 3.8 mmol/l). By contrast, the glycaemic thresholds for the onset of symptoms of hypoglycaemia (3.0 mmol/l) and the development of cognitive impairment (2.7 mmol/l) occur at lower blood glucose concentrations. Because the human brain is critically dependent on glucose as its principal source of fuel, acute hypoglycaemia results in impaired cognitive function with potential clinical consequences and many studies have examined the effects of hypoglycaemia on different cognitive domains (94,175). However, in humans, not all cognitive and motor functions are affected equally

during hypoglycaemia (Table 3.1). Complex tasks that require rapid decision-making, sustained attention and planning capabilities deteriorate during mild to moderate hypoglycaemia whereas simple cognitive and motor tasks are more resistant and remain relatively unaffected (94,175). A substantial degree of variability exists among the neuropsychological responses of individuals to hypoglycaemia in adult humans, whether or not they have type 1 diabetes (93-99,176,177). Some people develop severe cognitive impairment during hypoglycaemia, while others are unaffected by a similar decline in blood glucose concentration. The susceptibility of an individual to the detrimental effects of acute hypoglycaemia may be influenced by several different factors, and various moderators of cognitive performance during acute hypoglycaemia have been proposed.

The search for moderators that predict individual vulnerability assumes that this clinical response to hypoglycaemia is consistent across time and equivalent degrees of hypoglycaemia, and that it does not simply reflect individual variation within a population. Gonder-Frederick *et al* (178) have demonstrated that individual differences in cognitive responses to hypoglycaemia are consistent (178). In their study, 26 subjects with type 1 diabetes performed a battery of cognitive tests during euglycaemia (blood glucose 6.3 mmol/l), mild hypoglycaemia (blood glucose 3.6 mmol/l), moderate hypoglycaemia (blood glucose 2.6 mmol/l), and these were repeated following restoration of euglycaemia. To assess the temporal reliability of individual differences of performance deterioration during hypoglycaemia, 15 of the subjects were retested three months later. This study (178) demonstrated considerable variations between the subjects' responses to hypoglycaemia of varying

severity. At a blood glucose of 3.6mmol/l, 19% of the subjects exhibited a significant deterioration in cognitive performance, while almost half showed little or no deterioration. At a blood glucose of 2.6 mmol/l, a significant deterioration occurred in more than 50% of the subjects; only 15% showed little or no disruption in performance. On re-testing three months later, these individual variations in vulnerability to the development of cognitive dysfunction, and the degree of impairment that was observed in most tests, were very similar in individual subjects, indicating that the individual differences to hypoglycaemia were stable and did not occur at random. This would suggest that hypoglycaemia has no uniform effect on causing cognitive dysfunction, and infers that some individuals are more susceptible than others to the adverse effects of acute neuroglycopenia on neuropsychological function.

Table 3.1

Cognitive impairment is most evident in tasks requiring:
• Selective attention
• Rapid decision-making
• Analysis of complex visual stimuli
• Mental flexibility
• Memory of recently learned information
• Hand-eye co-ordination

MODERATORS OF COGNITIVE FUNCTION DURING ACUTE HYPOGLYCAEMIA

The factors that moderate the individual differences in glycaemic thresholds for the onset of hypoglycaemia-induced cognitive dysfunction are of clinical relevance to people with type 1 diabetes. Moderators that have been investigated include: age, gender, intelligence, the presence or absence of diabetes, the duration of diabetes, the magnitude of counterregulatory hormonal responses to hypoglycaemia, quality of glycaemic control, exposure to antecedent hypoglycaemia, the state of awareness of hypoglycaemia, a history of previous severe hypoglycaemia, the duration of hypoglycaemia and a variety of pharmacological agents.

Age

Age influences the counterregulatory responses to hypoglycaemia and modifies the symptoms that are experienced. With increasing age, the magnitude of responses of adrenaline and glucagons is lower, autonomic warning symptoms are less profound and neuroglycopenic symptoms predominate (18,179) (Figure 3.1). To examine the effects of ageing on the glycaemic thresholds for cognitive impairment, one study utilised a single test of cognitive function, the four-choice reaction time test, during controlled hypoglycaemia in two groups of non-diabetic men (17). One group consisted of seven older men between the ages of 60-70 years, and the second group comprised seven young men aged between 22-26 years. A hyperinsulinaemic glucose clamp was used to lower the blood glucose concentration from 5.0 to 2.4 mmol/l in a stepwise fashion, and the four choice reaction time, a test of general

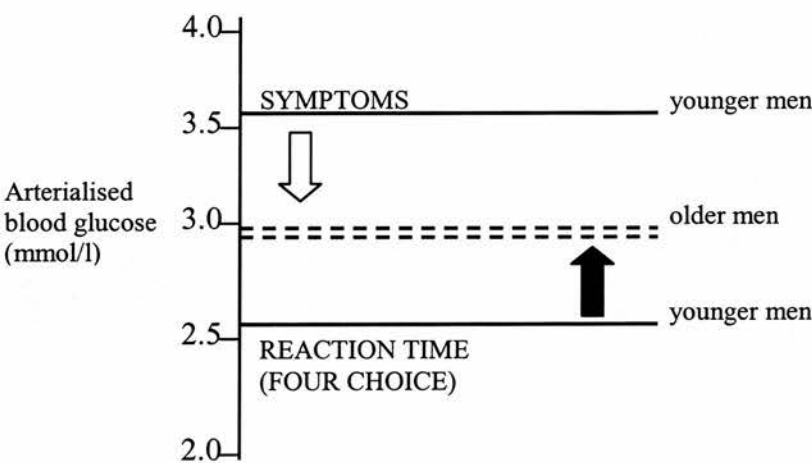
neuropsychological co-ordination and psychomotor speed, was administered. The counterregulatory hormonal responses to hypoglycaemia, symptoms of hypoglycaemia, and choice reaction time were assessed at each glucose plateau. The study demonstrated that cognitive function, as assessed by this single test, deteriorated at a higher blood glucose concentration (3.0 vs. 2.6 mmol/l), and to a greater degree, in the older group compared to the younger subjects. In the younger group, the symptoms of hypoglycaemia commenced at a higher blood glucose concentration (3.6 vs. 3.0 mmol/l) and were more intense, such that in the older men there was no perceptible difference between the blood glucose levels for the warning symptoms of hypoglycaemia and the development of cognitive impairment. This provides a very limited opportunity to detect the onset of hypoglycaemia and take appropriate action before more disabling neuroglycopenia supervenes. Thus, these age-related changes in the glycaemic thresholds for the symptomatic responses and for development of cognitive dysfunction during hypoglycaemia render older people at greater risk of not responding to hypoglycaemia and allowing progression to more severe hypoglycaemia.

In contrast to these findings (17), other studies that have examined the effect of age on cognitive function during hypoglycaemia have shown no consistent correlation between age and the degree of cognitive impairment during hypoglycaemia (97,98,178), although in these studies the age differences of the subjects was limited. In the study by Cox *et al* (97), only 10 people with type 1 diabetes were studied with ages ranging from 26-52 years of age, in the study by Draelos *et al* (98) the patients were aged between 18-44 years, and in the study by Gonder-Frederick *et al* (178) the patients were between 23 and 48 years of age. It is

probable that the age ranges of the subjects in these studies are too narrow to have allowed any significant effects of age on cognitive function during acute hypoglycaemia to have been elicited.

Figure 3.1

The difference between the glycaemic threshold for subjective awareness of hypoglycaemia and that for the onset of cognitive dysfunction may be absent in the elderly. Reproduced from Matyka *et al*, 1997 (17) with the permission of the American Diabetes Association.



Gender

Significant gender differences in cognitive performance during acute hypoglycaemia were demonstrated in a study of 20 men and 22 women with type 1 diabetes by Draelos *et al* (98), in which a wide range of cognitive abilities were tested. These included sensory perceptual processing, simple motor abilities, attention, learning and memory, language, and spatial and constructional abilities, which were examined at plasma glucose levels of 2.2, 5.6, 8.9, 14.4, and 21.1 mmol/l. All aspects of cognitive performance were impaired at a plasma glucose of 2.2 mmol/l when compared with the baseline performance at euglycaemia (plasma glucose 5.6 mmol/l). However, at this degree of hypoglycaemia cognitive impairment was less in women than in men in tests which examined selective and sustained attention and mental flexibility. This difference persisted after adjustment for other potential confounding factors. No gender differences were observed with tests of other aspects of cognitive function, nor was this discrepancy evident in the cognitive test scores obtained at lesser levels of hypoglycaemia. Other studies have shown that women have less intense counterregulatory hormonal and symptomatic responses to hypoglycaemia compared with men (180-182). Women may therefore be more able to tolerate hypoglycaemia than men and be more resistant to cognitive impairment.

The indication of a gender difference in cognitive susceptibility to hypoglycaemia is supported by a further study that demonstrated that women performed better on cognitive function testing than men during mild hypoglycaemia (venous plasma glucose 3.6 mmol/l) (178). However, in this study, the degree of

cognitive impairment was similar in both sexes during more profound hypoglycaemia (blood glucose 2.6 mmol/l).

Intelligence

It has been suggested that individuals with greater intelligence as measured by a higher IQ level, possess more 'brain reserve capacity' for cognitive processing that may be utilised if cognitive function is compromised, and that this will confer a degree of protection from various cerebral insults, such as hypoglycaemia (183). A study by Gold *et al* (184) sought to identify whether IQ level exerts a differential effect on the impairment of cognitive performance that is caused by acute hypoglycaemia. In this study, 24 healthy non-diabetic volunteers were divided into high and average IQ groups according to their performance in two standard tests of general intelligence (the Alice Heim 4 test and the National Adult Reading Test). Cognitive function was measured during euglycaemia (plasma glucose 4.5 mmol/l) and hypoglycaemia (plasma glucose 2.5 mmol/l). As anticipated, the high IQ group had a significantly better performance at baseline in most of the cognitive tasks. Cognitive function deteriorated during hypoglycaemia irrespective of IQ and very few differences in deterioration in performance were observed between the two groups. However, multiple univariate analysis of variance revealed an IQ effect on two of the cognitive tests. The group with average IQ deteriorated significantly less than the high IQ group during hypoglycaemia in tests of attention and information processing (Paced Auditory Serial Addition Test (PASAT) and Rapid Visual Information Processing). These results suggest that individuals with a higher IQ are not protected from the adverse effects of hypoglycaemia on cognitive function, and

that they may in fact be more vulnerable to the detrimental effects of neuroglycopenia on intellectual ability.

However, studies performed in people with diabetes have failed to demonstrate a relationship between intelligence (or a surrogate marker, such as the number of years of education) and changes in cognitive performance during hypoglycaemia (97-99,178).

Diagnosis of Diabetes

The question of whether the presence or absence of diabetes *per se* influences cognitive performance during hypoglycaemia was addressed in a study by Wirsén *et al* (185). Cognitive function was measured using a neuropsychological test battery before, during, and after acute insulin-induced hypoglycaemia (arterialised blood glucose 1.8-2.0 mmol/l) in 10 men with type 1 diabetes and in 12 non-diabetic men (185). The two groups were matched for age and educational level, and no differences between the two groups in neuropsychological test performance were apparent at euglycaemia. The reported symptoms of hypoglycaemia were comparable between the two groups, and significant deterioration of cognitive function occurred in both groups during hypoglycaemia. However, the group of subjects with diabetes demonstrated a greater degree of cognitive impairment during hypoglycaemia than the non-diabetic group. This would suggest that people with type 1 diabetes are more susceptible to the effects of neuroglycopenia. It is possible that this may represent some form of sub-clinical effect of diabetes on the brain (a diabetic 'encephalopathy'), either as a consequence of repeated exposure to severe hypoglycaemia or from the long-term effects of chronic hyperglycaemia (104,186).

However, no difference in cognitive function was observed between the two groups at baseline and the subjects with diabetes had higher blood glucose concentrations before the induction of hypoglycaemia, which may influence cognitive performance. The results of the study by Wirsén *et al* (185) should be interpreted with caution. It is possible that the difference in cognitive performance during hypoglycaemia that was observed between the two groups may have been related to the greater absolute reduction in blood glucose that was required to achieve a determined level of hypoglycaemia in the diabetic group.

In contrast to these observations, no differences between diabetic and non-diabetic subjects were noted in cognitive performance during acute hypoglycaemia in two earlier studies (96,152). In a third study which included diabetic and non-diabetic subjects (187), differences in cognitive performance between the groups were observed at baseline, which precluded interpretation of the effects of diabetes on responses during hypoglycaemia.

Other Diabetes-Related Clinical Variables

No relationship has been observed between the degree of cognitive dysfunction during hypoglycaemia and variables such as duration of diabetes (96,98,178,188), age of onset of diabetes (99,178) and the magnitude of counterregulatory hormonal responses to hypoglycaemia (96-98). In one study (178), a greater deterioration in cognitive performance during acute hypoglycaemia in people with type 1 diabetes was associated with a previous history of coma caused by severe hypoglycaemia.

Glycaemic Control

Strict glycaemic control alters the blood glucose thresholds at which the symptomatic and counterregulatory hormonal responses to hypoglycaemia are initiated, in that a lower blood glucose is required to trigger these responses (150,151). While it would seem likely that the threshold for the onset of cognitive dysfunction during hypoglycaemia would also be modified by the level of previous glycaemic control, this proposal has aroused controversy and the evidence is conflicting.

A study by Widom and Simonson (152) compared neuropsychological function during hypoglycaemia in a group of eight subjects with type 1 diabetes who had good glycaemic control (mean HbA1c 8.0%) and a group of nine subjects who had much poorer glycaemic control (mean HbA1c 11.8%). A battery of cognitive tests was administered during a hyperinsulinaemic glucose clamp in which blood glucose was lowered in a stepwise fashion from 5.0 to 2.2 mmol/l. The median glucose threshold for the onset of cognitive dysfunction did not differ statistically between those with better glycaemic control diabetes from those with poorer control. Similar results were observed by Maran and colleagues (164) in an examination of the glycaemic thresholds for the deterioration of four-choice reaction time during acute hypoglycaemia in eight subjects with intensively-treated type 1 diabetes (mean HbA1c 7.7%) and ten subjects on conventional therapy who had sub-optimal glycaemic control (mean HbA1c 10.1%) (164). Their results are in concordance with those of several other studies (95-97,176,177,183,189) that have failed to demonstrate any correlation between the quality of glycaemic control and cognitive performance during acute hypoglycaemia.

A link between glycaemic control and neuropsychological test performance has been demonstrated during acute hypoglycaemia in a study by Holmes and colleagues (176). In subjects with type 1 diabetes who had strict glycaemic control (mean HbA1c 6.9%), a significantly poorer performance in an auditory reaction time test was observed when compared with subjects with less strict glycaemic control (mean HbA1c 8.8%) (176). However, these results need to be interpreted with some caution as the subjects with strict glycaemic control had a history of experiencing a significantly greater number of episodes of coma caused by severe hypoglycaemia which may have influenced their performance and the number of subjects in the study (n=15) was small.

Neuropsychological studies that have examined the effects of acute hypoglycaemia on sensory evoked potentials (P300 event-related potentials) gave results that are consistent with the findings of Holmes *et al* (94). Measurement of event-related potentials provides an objective quantitative assessment of cognitive function. The P300 component of these potentials is generated endogenously when a subject is required to discriminate and memorise a specific task-specific stimulus. Its latency reflects the speed of information processing and correlates with attention and short-term memory. Ziegler *et al* (159) addressed the issue of whether the quality of preceding glycaemic control will modify cognitive function during acute hypoglycaemia by measuring P300 event-related potentials during hypoglycaemia. Eighteen people with type 1 diabetes were studied, seven of whom had good glycaemic control (mean HbA1c 6.3%) and 11 of whom were less well controlled (mean HbA1c 9.1%). No significant difference between the two groups was present at baseline with regard to P300 latency but the glycaemic threshold at which a

significant increase of P300 latency was first detected was 1.6 mmol/l in subjects with strict glycaemic control, and 3.5 mmol/l in those with poorer control. Comparable results have been reported in another study that examined the effect of hypoglycaemia on P300 potentials in people with intensively and conventionally-treated diabetes (160).

Antecedent Hypoglycaemia

The thresholds for counterregulatory hormonal responses to hypoglycaemia are attenuated by preceding exposure to hypoglycaemia (antecedent hypoglycaemia) (141,190). A number of studies have tried to identify whether the glycaemic threshold for cognitive dysfunction during hypoglycaemia also changes in response to antecedent hypoglycaemia. However, the impact of antecedent hypoglycaemia on cognitive function is difficult to quantify; different degrees and durations of antecedent hypoglycaemia have been studied, and the duration of the time period between the episode of antecedent hypoglycaemia and the subsequent hypoglycaemia study varies considerably between studies.

Mellman *et al* studied the effects of antecedent hypoglycaemia in nine non-diabetic volunteers (191). Antecedent hypoglycaemia (arterialised blood glucose 3.2 mmol/l) or euglycaemia (blood glucose 5.2 mmol/l) was maintained for two hours, 90 minutes before performing to a stepped hypoglycaemic clamp, during which cognitive function was assessed. Two tests of cognitive function were administered: Logical Memory, a test of immediate verbal memory, and the Digit Symbol Substitution Test, a test of general psychomotor performance. It was found that the Digit Symbol Substitution test scores deteriorated equally during hypoglycaemia,

irrespective of whether antecedent hypoglycaemia or euglycaemia had been present, but the performance during hypoglycaemia in the memory task was preserved following antecedent hypoglycaemia. This suggests that the preservation of Logical Memory function during hypoglycaemia following a recent episode of antecedent hypoglycaemia may have resulted from adaptation of the central nervous system to low blood glucose.

Asymptomatic nocturnal hypoglycaemia is a very common problem amongst people with type 1 diabetes (192,193). Veneman *et al* (42) and Fanelli *et al* (162) have studied the impact of antecedent nocturnal hypoglycaemia on cognitive function during hypoglycaemia the following day. Veneman *et al* measured cognitive function during hyperinsulinaemic hypoglycaemic glucose clamps in 10 healthy volunteers on two occasions; once after induction of asymptomatic nocturnal hypoglycaemia (plasma glucose 2.4 mmol/l) for two hours, and once after a euglycaemic control study in which saline instead of insulin was infused overnight (42). The deterioration of cognitive function during hypoglycaemia the following day was significantly less after the asymptomatic antecedent nocturnal hypoglycaemia, compared to antecedent nocturnal euglycaemia. In addition, the plasma glucose concentration at which cognitive dysfunction developed was significantly lower following antecedent hypoglycaemia. Fanelli *et al* (162) also demonstrated that a single episode of moderate asymptomatic antecedent nocturnal hypoglycaemia diminished cognitive dysfunction during a subsequent episode of controlled hypoglycaemia the following day. In this study, the effect of antecedent nocturnal hypoglycaemia (arterialised plasma glucose 2.8 mmol/l) was examined in fifteen people with type 1 diabetes. The subjects were exposed for 3.5 hours to either

nocturnal hypoglycaemia or euglycaemia. After nocturnal hypoglycaemia, overall cognitive dysfunction was less in comparison with nocturnal euglycaemia when cognitive tests were applied during hypoglycaemia induced on the following day.

The results of these three studies suggest that the glycaemic thresholds for hypoglycaemic cognitive dysfunction shift to lower plasma glucose concentrations after *recent* antecedent hypoglycaemia, both in non-diabetic people and in those with type 1 diabetes. By contrast, a study by Hvidberg *et al* (165) of 16 non-diabetic subjects reported that the glycaemic thresholds for cognitive dysfunction during hypoglycaemia did not shift to lower plasma glucose concentrations after a period of antecedent hypoglycaemia. In the afternoon, the subjects were exposed for two hours either to moderate hypoglycaemia (mean blood glucose 2.6 mmol/l) or, on a separate occasion, to euglycaemia. The following morning, cognitive function tests were administered during a hypoglycaemic clamp. No significant overall effect of exposure to antecedent hypoglycaemia was observed on subsequent hypoglycaemic cognitive dysfunction. However, when performance of individual tasks at specified blood glucose concentrations was considered, less deterioration in tasks of attention and pattern recognition was observed when plasma glucose concentrations were reduced from 2.8 to 2.5 mmol/l.

Several other studies have not shown any effect of antecedent hypoglycaemia on hypoglycaemia-induced neuropsychological dysfunction. Dagogo-Jack *et al* exposed 16 people with type 1 diabetes to either antecedent afternoon hypoglycaemia or antecedent afternoon euglycaemia (149). Cognitive function during hypoglycaemic clamps carried out on the following morning was not significantly affected by the antecedent hypoglycaemia. Indeed, some aspects of cognition

(attention) were actually worse following antecedent hypoglycaemia. In another study by George *et al* (194), eight people with type 1 diabetes were exposed to antecedent hypoglycaemia (arterialised blood glucose 2.8 mmol/l) or antecedent euglycaemia (blood glucose 5.0 mmol/l), which was followed, after an interval of two days, by a further episode of hypoglycaemia with use of the four-choice reaction time test. The results showed that the decrement in cognitive performance induced by hypoglycaemia was unaffected by exposure to antecedent hypoglycaemia. In addition, studies by Bendtson *et al* (195) and King *et al* (102) detected no effect of antecedent nocturnal hypoglycaemia on cognitive test performance the following day.

Finally, a study by Ovalle *et al* (161) investigated the impact of recurrent antecedent hypoglycaemia on subsequent cognitive performance during a stepped hypoglycaemic clamp. In this study, six patients with type 1 diabetes were exposed to either two hours of recurrent hypoglycaemia (blood glucose 2.8 mmol/l) twice a week for a period of one month, or to two hours of hyperglycaemia (8.3 mmol/l) twice a week for a month. Recurrent antecedent hypoglycaemia was found to cause a significant reduction in overall cognitive dysfunction during hypoglycaemia.

Duration of hypoglycaemia (short-term cerebral adaptation)

Cerebral adaptation can occur following exposure to low blood glucose concentrations resulting in a change in the glycaemic thresholds for secretion of counterregulatory hormones and for the generation of symptoms of hypoglycaemia to lower blood glucose levels (i.e. a more profound hypoglycaemic stimulus is required). The brain appears to have an inherent ability to adapt so as to be able to

function normally despite a diminished supply of glucose. The glycaemic threshold for the onset of cognitive dysfunction may also be affected by cerebral adaptation, but this remains uncertain. The putative mechanism underlying cerebral adaptation is unknown but may involve upregulation of glucose transporters which control the rate of glucose transporters into neurones.

In two studies by Kerr *et al*, performance in a single choice reaction time test was studied during prolonged hypoglycaemia in people with (196) and without (197) type 1 diabetes. Arterialised blood glucose concentration was clamped at 3.5 mmol/l for one hour then reduced to, and maintained at, 2.8 mmol/l (196) and 3.0 mmol/l (193) for 90 (196) and 60 (197) minutes, respectively. Reaction times were measured at baseline, and twice at the glucose plateaus. Counterregulatory hormone levels and symptomatic awareness of hypoglycaemia were also documented. The reaction times initially slowed when the blood glucose concentration was lowered from 3.5 mmol/l to the blood glucose nadir, compared to the baseline scores. However, after prolonged hypoglycaemia the reaction times improved towards baseline scores. The improvement in reaction time performance during prolonged hypoglycaemia occurred in parallel with a reduction of symptomatic awareness of hypoglycaemia. Meanwhile, counterregulatory hormone levels remained elevated during prolonged hypoglycaemia. Therefore, as hypoglycaemia continued, the symptom score declined and cognitive function improved, while levels of counterregulatory hormones remained elevated. Although alternative cerebral fuels have been shown to be used by the brain during hypoglycaemia (ketones, amino acids, free fatty acids), it is unlikely that the utilisation of ketone bodies could be used by the brain in these circumstances as ketone body formation would be

suppressed by the hyperinsulinaemic clamp. The findings of these studies (196,197) have been interpreted as demonstrating some degree of cerebral adaptation occurring during mild hypoglycaemia. However, this study is fundamentally flawed in that a separate euglycaemia study in the subjects studied indicated that there was a practice effect for the reaction time task, which therefore improved with increasing application. No statistical comparison of the results from the hypoglycaemia and euglycaemia clamps was reported. It should be noted that a blood glucose nadir of 2.8 or 3.0 mmol/l is not very low and that most cognitive functions become impaired at glucose levels below this.

In contrast to these findings, Gold *et al* found no evidence of cerebral adaptation in 24 non-diabetic subjects following exposure to 40-60 minutes of hypoglycaemia (arterialised blood glucose 2.5 mmol/l) (198). This is a much more scientifically robust study in that an extensive test battery was utilised, with statistical adjustment for potential practice effects by comparing the hypoglycaemia clamp results with those obtained during a separate euglycaemic clamp. However, the duration of hypoglycaemia was relatively short, and it is likely that a more prolonged period of exposure to hypoglycaemia is required to induce cerebral adaptation.

The effect of protracted, prolonged hypoglycaemia on cerebral adaptation was investigated by Boyle *et al* by exposing a group of 12 non-diabetic volunteers to hypoglycaemia for a period of four days (153). On the first day, a stepped hypoglycaemic clamp was performed, lowering blood glucose concentration from 4.72 to 2.5 mmol/l. At each plateau of blood glucose, symptomatic awareness of hypoglycaemia, counterregulatory hormone levels and cognitive function were

measured and brain glucose uptake was also calculated. The arterialised blood glucose concentration was maintained at 2.9 mmol/l for 56 hours. On the final day the stepped glucose clamp procedure was repeated, starting with a blood glucose concentration of 4.16 mmol/l, and the same measurements were repeated. The glycaemic thresholds at which subjects developed symptoms of hypoglycaemia and at which cognitive function declined occurred at much lower blood glucose levels during the final clamp compared to the results obtained on the first day. The threshold for counterregulatory hormone secretion was also modified. On the first day, brain glucose uptake became impaired at a blood glucose concentration of 3.61 mmol/l, but on the final day it remained preserved throughout the hypoglycaemic clamp. This would suggest that following prolonged exposure to a low blood glucose level adaptation had occurred within the brain to preserve and protect brain glucose uptake and maintain cerebral function during hypoglycaemia. However, the results of this study should be interpreted with caution since potential practice effects of the cognitive tasks were not evaluated, and the statistical analysis compared cognitive performance at each blood glucose level within a clamp with its own baseline. Interaction analyses between the clamps were not performed, thereby increasing the risk of a spurious effect emerging in line with the experimental hypothesis.

Impaired awareness of hypoglycaemia

Scientific assessment of the impact of impaired awareness of hypoglycaemia on cognitive function during acute hypoglycaemia is hampered to some extent by the lack of a validated, non-subjective means of defining symptomatic awareness.

Moreover, it is difficult to separate out the confounding influences of recent antecedent hypoglycaemia and of strict glycaemic control, which are inter-related. In a study by Heller *et al* (187), the effects of insulin-induced hypoglycaemia on subjective detection of hypoglycaemia and on cognitive function using the four-choice reaction time test were studied in 15 people with type 1 diabetes. Blood glucose was lowered progressively using a stepped hypoglycaemic clamp to 2.5 mmol/l, and maintained at this level for 30 minutes. Subjects were asked “Do you feel hypoglycaemic?” and a hypoglycaemia symptom questionnaire was applied. This showed that, at a blood glucose concentration of 2.5 mmol/l, only four of the fifteen subjects recognised that the blood glucose was low. The subjects were not matched for IQ, and those who had impaired awareness of hypoglycaemia had a longer duration of diabetes and a lower mean glycated haemoglobin concentration than the subjects who had normal awareness. Cognitive performance was affected to the same degree during hypoglycaemia in the subgroup of people with intact symptomatic awareness compared to the larger number with apparent impaired awareness. What this study shows is that the glycaemic threshold for generation of symptoms varies between individuals, and is influenced by glycaemic control and other factors such as duration of type 1 diabetes, but actual impairment of hypoglycaemia awareness was not demonstrated.

A recent study by Zammitt *et al* (199) has shown a reduced decrement in cognitive ability during hypoglycaemia in people with impaired awareness compared to subjects with normal awareness of hypoglycaemia. Twenty subjects with type 1 diabetes and normal self-assessed hypoglycaemia awareness (12 male, median HbA_{1c} 8.7%, median age 30 years) and 15 subjects with impaired hypoglycaemia awareness

(6 male, median HbA_{1c} 8.2%, median age 34) underwent two hyperinsulinaemic glucose clamps, during euglycaemia (blood glucose 4.5 mmol/l) and during hypoglycaemia (2.5 mmol/l). The hypoglycaemia-aware group suffered impairment of short-term verbal memory at 2.5 mmol/l, whereas the unaware group did not. This finding is consistent with cerebral adaptation having developed in the unaware group.

By contrast, Gold *et al* (125) demonstrated that people with type 1 diabetes with impaired awareness of hypoglycaemia (estimated using a validated self-rating scale (30) exhibited a trend towards greater cognitive dysfunction during acute hypoglycaemia, which persisted for longer following restoration of euglycaemia, compared to subjects with normal awareness (199). The aware and unaware subjects in this study were matched for pre-morbid IQ, duration of diabetes, HbA_{1c} and exposure to previous episodes of hypoglycaemia. There was, however, a difference in IQ between the two groups (0.5 standard deviation), with the unaware subjects having lower mean scores on the Alice Heim 4 task (a test of general intelligence). Although this difference was not statistically significant, it is possible that this difference may have confounded the study results.

Symptomatic awareness of hypoglycaemia may be restored in people who have impaired awareness of hypoglycaemia if hypoglycaemia is strictly avoided (77). The effect of the restoration of hypoglycaemia awareness on the glycaemic thresholds for the development of cognitive dysfunction during acute hypoglycaemia has been examined, and the results are conflicting. Cranston *et al* (78) investigated the effects of strict avoidance of hypoglycaemia on symptomatic, cognitive and hormonal responses to hypoglycaemia in 12 men with type 1 diabetes. The subjects

were aged between 28-55 years, had a duration of diabetes between 11 and 32 years, and all had impaired awareness of hypoglycaemia. Six subjects had good overall glycaemic control (mean HbA1c 6.5%) and six had relatively poor control (mean HbA1c 8.2%). After strict avoidance of hypoglycaemia for three consecutive weeks (which took a mean duration of four months to achieve), hormonal and symptomatic responses to controlled hypoglycaemia during a hyperinsulinaemic hypoglycaemic clamp increased in magnitude. However, the glycaemic threshold for cognitive dysfunction (assessed by performance in the four choice reaction time test) did not change, with a deterioration in cognitive function occurring at similar blood glucose concentrations (2.8 mmol/l) in all patients, both before, and after the period of hypoglycaemia avoidance. Contrary to these findings, in a study by Fanelli *et al* (79), restoration of awareness of hypoglycaemia was associated with a change in the glycaemic threshold for cognitive dysfunction during acute hypoglycaemia. Sixteen people with type 1 diabetes were studied, all of whom had impaired awareness of hypoglycaemia. Exposure to hypoglycaemia was avoided for a period of two weeks. Cognitive function during, and symptomatic awareness of, hypoglycaemia were measured during a stepped hypoglycaemic clamp before, and after the period of avoidance of hypoglycaemia. Counterregulatory hormonal and symptomatic responses to hypoglycaemia improved following hypoglycaemia avoidance. Moreover, the blood glucose concentration at which cognitive dysfunction commenced was higher following the period of avoidance of hypoglycaemia. In addition, the degree of cognitive dysfunction at a given glucose concentration was less (79). These changes were maintained following a year of hypoglycaemia

avoidance (79), and were also observed in subjects with impaired awareness of hypoglycaemia who had type 1 diabetes of short duration (80).

Mitrakou *et al* examined the effects of reversibility of hypoglycaemia unawareness on cognitive function during hypoglycaemia in people with insulinomas (200). Autonomic and neuroglycopenic symptoms, counterregulatory hormonal responses and cognitive function were measured during hypoglycaemia in six people with insulinomas using a stepped hypoglycaemic clamp before and approximately six months after curative surgery and in 14 normal subjects matched for age, sex and weight. Before surgery, the patients with insulinomas had lower scores than the control subjects for autonomic and neuroglycopenic symptoms, had impaired counterregulatory hormonal responses and exhibited less cognitive dysfunction compared to normal subjects during hypoglycaemia. Surgical cure reversed all these abnormalities.

Pharmacological agents

The effect of a various pharmacological agents on cognitive performance during acute hypoglycaemia has been examined, principally in non-diabetic volunteers..

Agents that alter membrane channels for potassium adenosine triphosphate (KATP) in pancreatic beta cells have been shown to improve cognitive function during acute neuroglycopenia. In a study of 10 healthy males (201), performance in the four-choice reaction time test deteriorated at a glycaemic threshold of 2.5 mmol/l during treatment with glibenclamide, compared to a glucose level of 3.0 mmol/l in subjects treated with diazoxide and 2.9 mmol/l in subjects treated with placebo (201).

Structural similarities exist between the pancreatic beta cell and glucose sensitive neurones within the hypothalamus, and sulphonylurea receptors have been found to be widely distributed in neuronal cells throughout the brain.

Evidence also exists that some aspects of cognitive function may be protected by the administration of alanine during acute hypoglycaemia. In a study by Evans *et al* (202), a hyperinsulinaemic glucose clamp was used to lower the concentration of plasma glucose to 2.5 mmol/l in the presence of an infusion of either intravenous alanine or intravenous saline in seven healthy male volunteers. Cognitive function, assessed by the Stroop colour-word test, deteriorated less in the presence of the alanine infusion compared to saline, although performance in several other cognitive tests showed no benefit. It is possible that during hypoglycaemia alanine can be utilised by the brain as a metabolic fuel instead of glucose, or can do so by enabling an increased availability of lactate.

Smith *et al* studied the effects of modafinil on cognitive function during acute hypoglycaemia (203). Modafinil inhibits the release of the inhibitory neurotransmitter gamma-aminobutyric acid (GABA). Nine healthy male volunteers were randomly assigned to take either 200mg of modafinil or a placebo, following which a hyperinsulinaemic glucose clamp was used to maintain euglycaemia or reduce plasma glucose to 2.4 mmol/l. Modafinil had no effect on cognitive performance during euglycaemia. However, during hypoglycaemia, performance in two cognitive function tasks (the Stroop colour-word test and simple reaction time) deteriorated less in the modafinil-treated group.

Alcohol and hypoglycaemia independently affect cognitive function adversely. Cheyne *et al* (204) have demonstrated that the combined effects of both

hypoglycaemia and alcohol are additive. In this study, a hyperinsulinaemic clamp was used in 17 healthy subjects who were studied during euglycaemia (blood glucose 4.5 mmol/l) with placebo, euglycaemia with alcohol, hypoglycaemia (2.8 mmol/l) with placebo and hypoglycaemia with alcohol. The blood alcohol concentration during the euglycaemia and hypoglycaemia arms was identical at 43 mg/dl. Cognitive function was assessed using the Four-Choice Reaction Time test, the Trail Making B test and the Digit Symbol Substitution Test. The administration of alcohol during euglycaemia was associated with a deterioration in performance in the Four-Choice Reaction Time and Trail Making B tests, and the hypoglycaemia and placebo arm was associated with a deterioration in performance in the Four-Choice Reaction Time test only. However, when alcohol was combined with hypoglycaemia the deterioration in performance was significantly greater in all of the cognitive function tests.

Several studies have also looked at the potential effect of caffeine on cognitive function during hypoglycaemia. Caffeine has been shown to augment the symptomatic and counterregulatory responses to hypoglycaemia, thereby increasing recognition of hypoglycaemia. However, with the exception of one study by Watson *et al* (205) who demonstrated only a small improvement in performance in a single cognitive test (the Four-Choice Reaction Time test) during hypoglycaemia, other studies have shown that caffeine has no effect on cognitive function (206-208).

Mood

The short-term impact of hypoglycaemia on mood has attracted much less attention than the effects on cognitive function. Acute hypoglycaemia has a

profound effect on mood (94,209-213). Changes in various emotions are common and this may influence cognitive performance during hypoglycaemia. Moderate hypoglycaemia may induce a state of anxious tension, unhappiness and low energy, and even irritability and anger. As for cognitive function *per se*, considerable interindividual differences are observed in the effects of hypoglycaemia on mood and it would appear that an idiosyncratic relationship exists in individual subjects between mood and a low blood glucose. It could be speculated that this discrepant effect between individuals may have a direct influence on their cognitive performance during acute hypoglycaemia.

CONCLUSIONS

Individual differences in susceptibility to the effects of acute hypoglycaemia on cognitive function may therefore be mediated by a large number of interacting factors (Table 3.2). Increasing age and male gender may make people more susceptible to the effects of hypoglycaemia on intellectual function and, less convincingly, the presence of diabetes *per se* and a higher intelligence may also confer a greater predilection for cognitive impairment in the presence of neuroglycopenia. However, for those who have type 1 diabetes, the duration of the disorder, the age of onset and the integrity of the hormonal counterregulatory responses to hypoglycaemia do not appear to be important determinants of the nature or degree of cognitive decrements during hypoglycaemia, and evidence exists that prolonged or repeated exposure to moderate hypoglycaemia induces cerebral

adaptation with a subsequent improvement in cognitive performance or no apparent effect on intellectual abilities at the same degree of hypoglycaemia.

Controversy continues to surround the impact of impaired awareness of hypoglycaemia, strict glycaemic control and antecedent hypoglycaemia on the ability of the brain to adapt to frequent exposure to neuroglycopenia. Some studies have supported the premise that the glycaemic thresholds for the impairment of differing domains of cognitive function are shifted to commence at lower blood glucose concentrations in people who are exposed to recurrent hypoglycaemia because of these conditions or circumstances in a manner analogous to the well-recognised shift in glycaemic thresholds for counterregulatory hormonal responses and the generation of warning symptoms. However, a shift in glycaemic thresholds for cognitive dysfunction has not been demonstrated in other studies in similar groups of patients. It is possible that methodological differences between the studies, and in particular small sample size giving insufficient statistical power, may be responsible for these discrepant results. After discounting investigations which have obvious methodological limitations, it is noticeable that those studies which have demonstrated a shift in cognitive thresholds have utilised either a battery of cognitive function tests or neurophysiological changes of sensory evoked potentials. By contrast, the studies that did not demonstrate any change in glycaemic thresholds have often utilised only a solitary or, at most, two cognitive tasks (175,212). It is likely that not all cognitive tests are affected to a similar degree by conditions such as impaired awareness of hypoglycaemia, strict glycaemic control or recurrent antecedent hypoglycaemia, so that very small batteries of cognitive tests are inadequate to demonstrate an effect on glycaemic thresholds (214).

The psychometric test performance impairments observed during experimental studies of acute hypoglycaemia are clinically relevant. While it is difficult to evaluate the direct relevance of most experimental cognitive tests to tasks performed in everyday life, driving is an important activity that involves many cognitive processes and has been studied during hypoglycaemia. Studies using a driving simulator have examined the effect of hypoglycaemia on driving performance, although results have been inconsistent between groups of investigators. For example, Hoffman *et al* found no effect of hypoglycaemia (venous blood glucose 2.7 mmol/l) on driving (80), while studies by Cox *et al* have consistently demonstrated that driving performance is significantly impaired when arterialised plasma glucose concentration declines below 3.8 mmol/l (76,215). In addition, they demonstrated interindividual differences in driving performance during hypoglycaemia which have been shown to be reproducible and stable on re-testing after an interval of three months. Moderators of cognitive performance during hypoglycaemia may have important implications for people with insulin-treated diabetes in relation to their performance at work which would become impaired if they were exposed to hypoglycaemia.

Table 3.2

Factors increasing degree of cognitive dysfunction during acute hypoglycaemia:

- Increasing age
 - Male gender
 - Presence of Type 1 diabetes
 - Recent antecedent hypoglycaemia
 - Impaired awareness of hypoglycaemia
 - High IQ
-

Chapter 4

Memory

Memory is the subject concerning the concepts that the brain employs to store and retrieve information, or the process referred to as remembering. It is the cognitive process of storing, encoding and retrieving information and pervades everyday functioning.

CLASSIFICATION OF MEMORY SYSTEMS

A useful classification recognised three main memory systems: sensory, short-term and long-term memory (216). *Sensory memory* is memory in which representations of the physical features of a stimulus are stored for a very brief time, perhaps for a second or less, and it is difficult to distinguish from the process of perception. Although the measurement of sensory memory presents practical problems, the effect of hypoglycaemia on this memory system has been examined previously (217-219). The function of sensory memory appears to be to retain information for a sufficient length of time for it to be transferred to *short-term memory*. Short-term memory refers to the function that temporarily retains stimuli that have just been perceived from the environment or retrieved from long-term memory. Its capacity is limited in terms of the number of items that can be stored. Short-term memory lasts for a duration measured in seconds. Information may be transferred from short-term memory to *long-term memory* by the process of consolidation (220). Through rehearsal, the neural activity corresponding to sensory stimulation can be sustained, which causes permanent structural changes in the brain that are responsible for the formation of long-term memory (221,222). Long-term memory refers to information that is represented on a permanent or near permanent

basis. Unlike short-term memory, long-term memory has no known limits and is relatively durable.

WORKING MEMORY

Memory has been clinically and experimentally well studied, and has been shown to involve brain regions such as the hippocampus. It is thought to be mediated by changes in cell functioning. Early psychological work in the 1950's and 1960's led to the hypothesis of 'short term memory'; a process of limited capacity and only operative over a few seconds. The concept of 'working memory' is an extension of this idea, with the added idea that short term memory is woven together with higher cognitive processes, such as learning, reasoning, and comprehension.

Working memory, a short-term memory system which allows the retention and manipulation of information concurrently, is vital for daily activities. Working memory comprises a *phonological loop* (a store of phonetic, verbal information), a *visuospatial scratchpad* (a store of spatial information and memories for movement), and a *central executive*, which supervises and updates the content of working memory (223-225). Working memory allows the short-term retention and manipulation of material. Working memory, therefore, is used to think about what is already known and to come to conclusions on the basis of that knowledge. It is used, for example, to remember what has been said at the beginning of a sentence and retain this until the sentence has been completed, it allows a telephone number to be remembered long enough for it to be dialled, and enables the calculation of simple mental arithmetic. Multivariate modelling and computer simulation studies show

that working memory is almost indistinguishable from reasoning, and that individual differences in working memory correlate almost perfectly with individual differences in the general factor derived from a battery of psychometric intelligence tests (226-229).

Unlike long term memory, which has a large clinical body of research, working memory has only recently become the focus of intense clinical study. It is often assayed in intelligence or cognitive examinations using span tests, in which patients are asked to repeat a set of digits in reverse order (if I read "8-9-3-2-1-9", you would say "9-1-2-3-9-8") or repeat in the correct alphabetical order a group of words that had been read aloud. Studies indicate that working memory is not one process; rather, it is made up of several separable processes.

The simplicity and fundamental nature of working memory systems, and the adaptability of working memory experiments, make it suitable for new brain imaging technologies. Both Positron Emission Tomography (PET) and Functional Magnetic Resonance Imaging (fMRI) capitalise on properties of cerebral blood flow to make inferences about underlying neural activity. Founded upon Baddeley's model of working memory, investigators have begun to explore neural correlates of working memory. Several neuroimaging studies provide evidence for a distinct neurological basis for a phonological loop, as well as separate processes for storage of items and retrieval (230). During the storage phase of verbal working memory tasks, activity is found in Broca's area (involved in speech production) in addition to supplementary and premotor areas (involved in movement) in frontal cortex, and is strongly consistent with activity in areas involved in preparation of speech from other

neuroimaging studies. In addition, different networks are involved in retrieval as compared with storage in the left lateralised frontal cortex (230,231).

The neural correlates of spatial or object storage, in pursuit of the visuospatial scratchpad, is somewhat more tenuous. Neuroimaging studies yield that there are different areas activated in spatial or object memory tasks compared to those in verbal working memory tasks. Neuroimaging studies also suggest a difference in storage systems compared with retrieval systems in spatial or object working memory, indicating that there are again separate networks at work (230).

The most fascinating line of inquiry confronts the idea of a 'central executive', a control system that mediates attention and regulation of processes occurring in working memory. The idea of a central executive was first postulated by Baddeley (224). Many investigators have seen evidence supporting the idea of a central executive; they have observed higher cognitive activity in an area in the prefrontal cortex, called DLPFC (Dorsolateral Prefrontal Cortex), during difficult tasks (231). This area shows activity during object working memory, and what are termed 'executive processes', such as planning, focusing attention on an object, switching between tasks, and 'inhibition' of short term storage. One powerful design to study executive processes is to tax working memory systems to its capacity, or to present the subject with two tasks to perform simultaneously. An example of such a difficult task is the Stroop task, where colour names are presented in different colours ("red", for example, might be presented in green text). Neuroimaging studies using these difficult tasks support the notion of a central executive control system (230). In addition to neuroimaging studies, there is converging evidence from animal models

and cellular studies. Electrophysiological studies of monkeys allow the interconnections of individual circuits to be mapped out. Many of these circuits are focused in the prefrontal cortex (232).

The pharmacology of working memory also proves fascinating. Dopamine, a largely inhibitory neurotransmitter with many functions, is thought to play a major role in working memory (233,234). The frontal cortex has many dopaminergic pathways, which may modulate the activity of the pyramidal cells in the frontal cortex (233). Again, although much evidence has been conducted in monkeys, the relationship to human working memory systems is unclear.

Despite the current intensive inquiry into how we remember things on a short time scale, the components of working memory remain poorly understood. Further research on the systems of working memory will result in greater understanding of this fundamental system that we use almost every moment of our lives, providing insights into the higher cognitive processes that it feeds.

Chapter 5

Hypotheses for Studies

STUDIES 1 AND 2

Acute hypoglycaemia is a common side effect of treatment with insulin in people with diabetes (1). The non-physiological doses of insulin that are used in standard treatment regimens often leads to a mismatch between blood glucose and plasma insulin concentrations, resulting in hypoglycemia. A recent study has confirmed the high frequency of hypoglycemia amongst people with type 1 diabetes (33). The mean rate of mild hypoglycaemia was 2.0 episodes/patient/week, and of severe hypoglycaemia was 1.3 episodes/patient/year.

The human brain is dependent on a continuous supply of glucose as its main source of energy. Cerebral deprivation of glucose, therefore, rapidly causes cognitive dysfunction through the direct effects of acute neuroglycopenia (106,235). Early observations noted that blood glucose concentrations below 3.0 mmol/l, as a consequence of either natural glucose fluctuations (236) or bolus injection of insulin (237), were associated with impaired motor co-ordination and mental speed. Since then, a large literature on the cognitive effects of acute hypoglycaemia has developed with recent interest focusing on the particular cognitive domains that are affected and the clinical relevance of the cognitive decrements that occur.

Two main techniques have been employed to induce hypoglycaemia experimentally: the insulin infusion technique and the hyperinsulinaemic glucose clamp. The former involves the intravenous infusion of insulin at various rates to reach the desired blood glucose concentration, while the hyperinsulinaemic clamp technique employs a fixed intravenous infusion rate of insulin with a variable intravenous infusion of dextrose to maintain blood glucose concentrations at the

determined level (238). However, in many clamp studies, a common methodological error has been to induce hypoglycaemia by glucose clamp using a stepwise decline in blood glucose with tests of cognitive function being administered during the final 30 minutes of each blood glucose plateau. This allows a practice effect to occur, which is confounded with the level of hypoglycaemia.

Hypoglycaemia affords the opportunity to study cognitive processes under controlled deprivation of the brain's energy supply. Memory is one of the most important cognitive domains with respect to everyday function, yet it has seldom been examined in detail during acute hypoglycaemia. To our knowledge, the effect of hypoglycaemia on this key cognitive area has not explicitly been studied previously, and there are no systematic studies involving short and longer-term memory processes. There is a particular lack of information concerning the effect of hypoglycaemia on working memory.

The present studies were designed to investigate the effects of experimentally induced hypoglycaemia on verbal and non-verbal tests of short-term, delayed and working memory in people with and without Type 1 diabetes. It was hypothesised that performance in memory tasks would be significantly impaired during acute hypoglycaemia in both diabetic and non-diabetic subjects.

STUDY 3

Many studies have demonstrated the effects of acute *hypoglycaemia* on cognitive function and on mood states, both in non-diabetic subjects and in people with type 1 and type 2 diabetes mellitus. In contrast, considerably less information is available on the effects of acute *hyperglycaemia* on cognitive function and non-cognitive functions, such as mood. Anecdotal descriptions by patients with either type 1 or type 2 diabetes suggest that altered mood (such as increased irritability), diminished well-being, and difficulties with rapid cerebration, occur when blood glucose is elevated.

Published data is contradictory. An early study by Holmes *et al* (239) examined verbal skills in 12 adults with type 1 diabetes under conditions of controlled euglycaemia, hypoglycaemia (blood glucose concentration 3.0 mmol/l) and hyperglycaemia (blood glucose 16.6 mmol/l). Word meaning and spelling skills were significantly disrupted during hypoglycaemia, and a trend towards poorer performance was observed during hyperglycaemia. A further study in 12 children with type 1 diabetes (240) with a mean age of 12.4 years also demonstrated impairment of cognitive function during acute hyperglycaemia. At a blood glucose concentration of 20-30 mmol/l, there was a reduction in performance IQ of 9.5% compared to euglycaemia.

Other studies do not support these data. A study of adolescents with type 1 diabetes (241) did not show any effect of hyperglycaemia on cognitive function, but the assessment was limited to tests of reaction time and trail making. In a cohort of 42 adult patients with type 1 diabetes mellitus, under conditions of controlled

hyperglycaemia (blood glucose 8.9, 14.4 and 21.1 mmol/l in a stepped hyperglycaemic insulin clamp), neither cognitive function (99), nor self-reported mood (242) was significantly affected. However, it should be noted that the study cohort had poor metabolic control (mean total HbA1 10.1%). Cerebral adaptation to low blood glucose concentrations is a recognised phenomenon (167). There may also be potential for cerebral adaptation in response to high prevailing blood glucose concentrations, and acute hyperglycaemia in people with poor glycaemic control may have different effects.

The short-term effects of hyperglycaemia on cognitive function and mood are therefore an area worthy of further study, with implications for effects of postprandial hyperglycaemia which is common in most patients treated with conventional diabetes therapies, including insulin and oral hypoglycaemic agents.

The aim of this study was to test the hypothesis that, in people with type 2 diabetes mellitus, acute hyperglycaemia compared to euglycaemia will adversely affect cognitive function and/or mood as measured by a medium effect (i.e. a mean difference of 0.5 SD) on a range of validated tests.

STUDIES 4, 5 AND 6

People with Type 1 diabetes are frequently exposed to acute hypoglycaemia, which in humans is associated with profound haemodynamic changes, including an increase in heart rate and stroke volume, increased myocardial contractility and a rise in cardiac output (48,50). Changes in peripheral blood pressure include an increase in systolic and a decrease in diastolic pressure, with no change in mean arterial pressure. The effects on central arterial pressure are not known.

Arterial blood pressure is usually recorded non-invasively at the brachial artery by sphygmomanometry. It is assumed that the pressure is the same throughout the arterial tree and that it represents an accurate index of aortic peak pressure and of left ventricular systolic pressure, and so of left ventricular afterload. However, blood pressure measured in the peripheral circulation is not an accurate estimate of central pressure because of amplification of the pressure pulse between central and peripheral arteries (244,245). Normally, there is amplification of the pulse pressure between the aorta and brachial artery (246). It is the aortic, rather than the brachial, pressure which determines left ventricular workload (247,248).

Central arterial pressure and arterial wall stiffness, which are independent predictors of cardiovascular morbidity (249-253), can be studied non-invasively using pulse wave analysis (254-257), which requires the external application of a micromanometer-tipped probe over a peripheral artery (258,259). Contraction of the left ventricle generates a pressure wave which travels along the major arteries until it meets sites of resistance (high resistance arterioles), then the wave is reflected back to the heart (260). The stiffness of the arterial wall influences the velocity of the

reflected wave. In healthy young people, the reflected wave reaches the heart during diastole and, by increasing diastolic pressure, enhances coronary perfusion. However, when arterial stiffening has developed, the increased pulse wave velocity of the stiffened arteries leads to earlier reflection of the wave so that it returns to the heart during late systole (249,261). The increase in systolic pressure that results from this earlier reflection of the pressure wave is known as 'augmentation', with augmentation index (AIx) measured as the augmented pressure expressed as a percentage of the pulse pressure. AIx increases with age, and in disease states such as hypertension (262) and hypercholesterolaemia (263). It is also influenced by heart rate; a decrease in AIx is observed with an increase in heart rate (264). Central arterial pressure and waveform convey important information about cardiovascular status (249,265,266).

The present study sought to measure arterial wall stiffness and the central haemodynamic responses to the administration of intravenous insulin and the induction of acute hypoglycaemia in people with Type 1 diabetes of varying duration and in non-diabetic subjects. It was hypothesised that increased duration of type 1 diabetes would be associated with increased central arterial wall stiffness.

PART II

METHODS

Chapter 6

Methods

Hyperinsulinaemic Glucose Clamp

In studies 1, 2 and 3 a hyperinsulinaemic glucose clamp was used to control blood glucose concentrations (238).

Each session commenced at 08.00 hours following a 10-12 hour overnight fast. An intravenous cannula was inserted retrogradely into a vein on the dorsum of the non-dominant hand for regular blood sampling. The hand was placed in a heated blanket to arterialise the venous blood. A second intravenous cannula was inserted into a vein in the antecubital fossa of the same arm for infusion of human soluble insulin (Humulin S; Eli Lilly) and 20% dextrose. Intradermal lignocaine (1%) was used for insertion of each cannula. Insulin was infused at a constant rate of 60 mU/m²/min using an IMED Gemini PCI pump (Alaris Medical Systems, San Diego, USA). A variable intravenous infusion of 20% dextrose was given simultaneously. The rate of dextrose infusion was varied according to the arterialised blood glucose concentration. This was measured at the bedside using the glucose oxidase method (Yellow Springs Instrument 2300 Stat, Yellow Springs, Ohio, USA). Blood glucose concentration was measured every three minutes initially and then every five minutes once a stable level had been achieved.

In studies 1 and 2, the arterialised blood glucose concentration was stabilised initially at 4.5 mmol/l (baseline) for a period of 30 minutes. In the euglycaemia condition, the blood glucose concentration was thereafter maintained at 4.5 mmol/l throughout the study. In the hypoglycaemia condition, blood glucose was lowered over a 20 minute period to 2.5 mmol/l. The blood glucose concentration was maintained at the predetermined target level for a further 10 minutes before

commencing the cognitive tests, and was maintained at this level for a further 70 minutes while the tests were administered. At the end of the hypoglycaemia study, the blood glucose level was restored to 4.5 mmol/l. Subjects were provided with a meal after completion of each study session.

In study 3, in each study condition the arterialised blood glucose concentration was stabilised initially at 4.5 mmol/l (baseline) for a period of 30 minutes. In the euglycaemia condition, the blood glucose concentration was maintained thereafter at 4.5 mmol/l throughout the study. In the hyperglycaemia condition, blood glucose was raised over a 20 minute period to 16.5 mmol/l. The blood glucose concentration was maintained at the predetermined target level for a further 10 minutes before commencing the cognitive tests, and was maintained at this level for a further 80 minutes while the tests were administered. At the end of the hyperglycaemia study, the blood glucose was restored to 4.5 mmol/l. Subjects were provided with a meal after completion of each study.

Cognitive Function Tests

In studies 1, 2 and 3, tests of general cognitive function were administered to the subjects. These included the Trail Making B and Digit Symbol Substitution tests, and the Reaction Time test (study 3 only).

General Cognitive Tests

Trail Making B

This is a test of complex visual scanning with a motor component, measuring visual conceptual and visual motor tracking. The subject is presented with a grid containing randomly positioned numbers and letters. The subject must connect consecutive numbers in numerical order and consecutive letters in alphabetical order, alternating between numbers and letters (267). The score is the time taken to complete the task.

Digit Symbol Substitution Test

This is a test of motor persistence, sustained attention, response speed and visuo-motor co-ordination. It consists of four rows containing, in all, 100 small blank squares, each paired with a randomly assigned number from one to nine. Above these rows is a printed key that pairs each number with a different symbol. The subject is asked to fill in as many of the blank squares with the appropriate symbol that matches the number above the box, in a time limit of 120 seconds (268). The score is the number of squares that are successfully completed within the 120 second time limit.

Reaction Time Test

This test is a test of psychomotor speed and information processing. It measures individuals' reaction time to a specific stimulus. In study 3, reaction time

was measured using a portable device, originally designed for the UK Health and Lifestyle survey (269). This has a LCD screen at the top with five response keys arranged below. Two versions of the test were used. In the Simple Reaction Time test, a zero was presented on the LCD screen at random intervals. Subjects were instructed to press the response key as quickly as possible when they saw the zero. In the Four-Choice Reaction time test, numbers 1, 2, 3 or 4 were presented randomly on the screen. Subjects were required to press the key corresponding to the number as quickly as possible. The time interval between a response and the display of the next digit varied randomly between 1 and 3 seconds for both Simple and Four-Choice Reaction Time tests. The mean scores for the tests were recorded in milliseconds.

Memory tests

The following memory tests were used:

Verbal Memory Tests

Auditory Verbal Learning Test (AVLT) – immediate and delayed

The AVLT is a test of immediate memory span, retrieval efficiency and learning. The delayed component measures longer-term retention (270). It consists of a list of 15 random words that are read to the subject at a rate of one word per second. The subject is asked to try and immediately recite the list as accurately as possible. This procedure is repeated five times. The sum total of words remembered correctly is designated the “immediate” score. Each subject is instructed to try to remember the list and is informed that recall of the list will be requested after a

period of one hour. The “delayed” score is the number of words that are recalled correctly at that time.

Logical Memory Test – immediate and delayed

Logical memory is a test of verbal learning. It measures immediate free recall following auditory presentation, and delayed recall (271). The subject is asked to recount a short story immediately after it is read to them, to remember the story and to recall it again after a delay of one hour. The story incorporates a number of specific points, each of which the subject must remember to achieve a score. The immediate and delayed scores are the sum total of the number of points remembered by the subject during immediate and delayed recall.

Visual Memory Tests

Visual Reproduction – immediate and delayed

This test measures immediate and delayed recall following visual presentation (271). Five designs (line drawings) are presented to the subject in sequence. The subject is allowed to study each design for ten seconds. The design is then removed and the subject is instructed to draw the design from memory. They are asked to draw the designs again after a delay of at least one hour. The designs are scored according to their accuracy. The total score is the sum of the scores of all five designs. Immediate and delayed scores are calculated separately.

Benton Visual Retention Test (BVRT)

This is a test of immediate visual recall. A series of ten designs of increasing complexity are presented to the subject, the designs are removed and the subject is

then asked to draw the designs from memory (272). The test is scored according to the accuracy with which the designs are drawn. The total score is the sum of the scores of all ten designs.

Working memory Tests

Digit Span – Forwards and Backwards

In the Digit Span test, a series of lists of random numbers is presented verbally to the subject, who must then recall the numbers in ascending numerical order (forwards) or reverse order (backwards) (271). The test score is the number of lists that are remembered correctly.

Letter/Number Sequencing

In the Letter/Number Sequence test, a series of lists of random numbers mixed with random letters is presented verbally. The subject must recall the list, stating the numbers in ascending numerical order and the letters in alphabetical order (271). The test score is the number of lists that are remembered correctly.

Four-term Order

Subjects are presented verbally with three commands, each command determining the sequence of four separate words. Subjects must arrange the words in the correct order according to the commands. Eight appropriate combinations of the four words are then shown to the subjects for 15 seconds. One option lists the words in the correct order; the other seven options are incorrect. Subjects are required to try to identify the correct option. Twenty-four problems, each with three commands and eight possible options, are presented to the subjects. The score achieved is the number of problems that are answered correctly (273).

Validation Span

In this test from Kyllonen's Cognitive Abilities Measurement battery (273), the subject is presented visually with a simple arithmetical problem. The subject is required to perform the calculation and to determine if the sum is correct or incorrect. On the left side of the page, adjacent to the problem, is placed an isolated and unrelated number. In addition to performing the simple mental arithmetic, the subject must remember this isolated number. Each problem is presented for five seconds. The problems are presented in sets of three, four and five. After completion of each set, the subject must recall the isolated left-sided numbers in the correct order. The score is based on how many of the isolated numbers are remembered correctly. An example of a set of three is given below:

$$5 \quad 3+2-1=4 \quad (\text{correct})$$

$$2 \quad 1-5+8=7 \quad (\text{incorrect})$$

$$3 \quad 2+4+6=12 \quad (\text{correct})$$

For this example, the correct sequence of numbers is 5,2,3.

Parallel versions of the tests are available for Logical Memory, AVLT, Benton Visual Retention Test, Four-term Order and Validation Span, and these were used to minimise a learning effect between the study conditions. Throughout the studies, tests were carried out in a predetermined fixed order.

Tests of Attention

In study 3, the Test of Everyday Attention (TEA) battery (274) was used to measure attention. It gives a broad-based measure of the most important clinical and

theoretical aspects of attention, and is the only test of attention based on everyday materials.

Map Search

This is a test of visual selective attention. The subjects had to search for a particular symbol, such as a sign that represented a garage, on a color map. The score is the number of symbols circled out of a possible maximum of 80 in two minutes. After the first minute had elapsed, the subjects were given a different colored pen to circle the map signs, to enable the number of targets located in the first minute to be identified and compared to the final total achieved.

Elevator Counting

This subtest was presented on audio-tape, and consisted of a simple counting procedure. It is a test of sustained attention. Subjects were asked to pretend that they were in an elevator whose floor-indicator was not functioning. They had to ascertain at which 'floor' they had arrived by counting a series of tape-presented tones.

Elevator Counting with Distraction

This subtest is similar to Elevator Counting, and was also presented on audio-tape. It differed in that subjects had to count the same tone that they had heard previously, while ignoring a distracting tone which had a higher note. It is a test of auditory selective attention.

Visual Elevator

In this subtest, the subject was asked to imagine that they were traveling up and down in an elevator, which in this case was represented by a series of pictures of elevator doors. Large arrows showed the direction of counting between pictures.

This reversal task is a measure of attentional switching, and hence of cognitive flexibility.

Elevator Counting with Reversal

This subtest was the same as the visual elevator subtest except that it was presented at a fixed speed on audio-tape and is another test of auditory selective attention.

Telephone Search

In this test, a test of visual selective attention, the subjects had to look for key symbols while searching through pages in a telephone directory.

Telephone Search while Counting

In this task, the subject had again to search for key symbols in a (different) telephone directory while simultaneously counting a series of strings of tones presented on audio-tape. By combining the scores for this subtest, and the time per target score for the previous telephone search, this test aims to provide a measure of divided attention, a 'dual task decrement'. In the factor analysis of normal data, this task loads on the sustained attention factor.

Lottery

In this final subtest of sustained attention, subjects had to listen for their (predetermined) winning lottery numbers. To do this, they had to listen to a 10 minute series of audio-tape-presented numbers of the form 'BC143' i.e. two letters followed by three numbers. The task was to write down the two letters preceding all lottery numbers ending in certain numbers e.g. '55'.

Parallel versions of the TEA battery are available and were used to minimise a learning effect between study conditions. Throughout the studies, tests were carried out in a predetermined fixed order.

Mood Questionnaire

In study 3, the UWIST mood adjective checklist, a validated subjective self-rating questionnaire, was used to document changes in mood experienced by the subjects during the euglycaemia and hyperglycaemia (275). There are three main mood states: *energetic arousal* (feeling lively and active versus tired and sluggish), *tense arousal* (feeling anxious and nervous versus relaxed and calm), and *hedonic tone* (feeling happy versus sad). The questionnaire consists of a list of 24 symptoms (e.g. cheerful, anxious, energetic). Each symptom can be categorized to one of the three main mood states. Each mood state is represented by eight different symptoms. The subject is required to score each symptom as Definitely, Slightly, Slightly Not, or Definitely Not (scoring 4, 3, 2 and 1 points respectively), to reflect how they are feeling at that precise time. From this, the total score for each mood state can be calculated.

Symptoms of Hypoglycaemia

In studies 1 and 2, the Edinburgh Hypoglycaemia Scale (276), a validated subjective self-rating questionnaire, was used to document the symptoms of hypoglycaemia experienced by subjects during the two studies. The symptoms of hypoglycaemia were classified as autonomic (hunger, palpitations, sweating, shaking), neuroglycopenic (drowsiness, confusion, inability to concentrate, speech difficulty, blurred vision) and non-specific (nausea, headache). Each symptom was graded on a Likert Scale of 1 to 7 (1=not present, 7=very intense).

Statistical Analysis

In studies 1, 2 and 3, the results were analysed independently for each cognitive test. A general linear model (repeated measures analysis of variance) was used with order of session (euglycaemia-hypoglycaemia or hypoglycaemia-euglycaemia for studies 1 and 2, euglycaemia-hyperglycaemia or hyperglycaemia-euglycaemia for study 3) as a 'between subjects' factor, and condition (euglycaemia or hypoglycaemia for studies 1 and 2, and euglycaemia or hyperglycaemia for study 3) as a 'within subjects' factor. A p value < 0.05 was considered to be significant. Effect size was calculated using the Eta squared test. All analyses were performed using SPSS version 10.0 for Windows.

PART III

HYPOGLYCAEMIA AND MEMORY

Chapter 7

Multiple Memory Functions are Impaired During Acute Hypoglycaemia in Non-Diabetic Humans

INTRODUCTION

The human brain is dependent on a continuous supply of glucose as its main source of energy. Cerebral deprivation of glucose rapidly causes cognitive dysfunction through the direct effects of acute neuroglycopenia (235). Acute hypoglycaemia is a common side-effect of treatment with insulin in people with diabetes, and can adversely affect their everyday activities (171,277).

In humans, controlled hypoglycaemia can be induced experimentally using the hyperinsulinaemic glucose clamp technique (238) and the effects of acute hypoglycaemia on cognitive performance has been examined in non-diabetic and diabetic subjects. Tasks that are complex, attention demanding and require a rapid response are more greatly impaired during neuroglycopenia, compared with simple cognitive and motor tasks, which are relatively preserved. In general, tests that involve attention, concentration, psychomotor skill, the accessing of long term memory and the ability to ignore distracting information, tend to deteriorate when blood glucose declines below 3.0 mmol/l (6,82,152,185,196,278).

Memory is one of the most important cognitive domains, that has seldom been examined during acute hypoglycaemia. Working memory is particularly important and the effects of acute hypoglycaemia on this domain have never been examined previously..

Previous studies of acute hypoglycaemia have used a heterogeneous assortment of memory measures as part of a larger battery of cognitive tests. Published studies suggest that short-term memory, as tested by word and story recall, can deteriorate

during hypoglycaemia (93,99,152,185,279). Other tests which assess memory can be identified in cognitive batteries that have been used to study the effects of acute insulin-induced hypoglycaemia in subjects with, and without, diabetes. Some studies have shown impaired memory functions (6,93,99,185,279), whereas in others, tests of memory were unaffected (152,239,280). Variable results may relate to methodological differences between studies. Many of the studies previously reported suffer from small sample size, application of a limited selection of (often obscure) tests, an inadequate ascertainment of biomedical and psychosocial variables that may affect the results, which have then been over-interpreted. In addition, the method of induction of hypoglycaemia has varied between studies, and the method of blood sampling (e.g. arterialised or venous blood) has differed. A common methodological error has been to induce hypoglycaemia by glucose clamp using a stepwise decline in blood glucose with tests of cognitive function being administered during the final 30 minutes of each blood glucose plateau, so allowing a prominent practice effect to occur. As a result of these problems, definitive evidence regarding the effects of acute hypoglycaemia on learning and memory is not available.

The present study was designed to investigate the effects of experimentally-induced hypoglycaemia on short-term, delayed and working memory in humans.

METHODS

Subjects

Sixteen (9 male) non-diabetic healthy volunteers were studied. Their median (range) age was 29.2 years (26.8-34.2) with a median Body Mass Index of 23.0 (20.2-24.1) kg/m². None of the participants had a history of chronic disease, previous head injury, seizure, blackouts, psychiatric illness or a family history of diabetes. They had no intercurrent illness and were taking no regular medication (with the exception of the oral contraceptive pill). Ethical permission for the study was approved by the Lothian Research Ethics Committee. All subjects gave written informed consent for the study.

Study Design

Each subject participated in two laboratory sessions, representing two different experimental conditions that were separated by at least two weeks (Figure 7.1). The studies were conducted in the Department of Diabetes at the Royal Infirmary of Edinburgh. A modified hyperinsulinaemic glucose clamp was used to maintain the blood glucose at a predetermined level (238). In the *euglycaemia* (E) condition, the arterialised blood glucose concentration was maintained at 4.5 mmol/l and hypoglycaemia was not induced. In the *hypoglycaemia* (H) condition, the glucose concentration was lowered to 2.5 mmol/l. The subjects were not informed which experimental arm of the study was being performed on each occasion and underwent the two experimental sessions in a randomised and counterbalanced fashion.

Procedure

Hypoglycaemia was induced using the hyperinsulinaemic glucose clamp procedure (238), as discussed in Chapter 6.

Cognitive Function Tests

Tests of immediate and delayed verbal memory, immediate and delayed visual memory, and tests of working memory were administered during the study conditions. In addition to the memory tests, the Trail Making B and Digit Symbol tests were also administered. These are validated tests of general cognitive ability and were used to confirm the effect of hypoglycaemia on cognitive function, as has been shown in previous studies (93,185,217,218,281).

Symptoms of Hypoglycaemia

The Edinburgh Hypoglycaemia Scale (276) was used to document the common symptoms of hypoglycaemia experienced by the subjects during the two studies.

RESULTS

A stable blood glucose plateau was achieved during both study conditions (Figure 7.2). The mean (SD) blood glucose concentration during the euglycaemia condition was 4.51 (0.21) mmol/l, and during the hypoglycaemia condition was 2.46 (0.11) mmol/l. Statistical analysis revealed that no significant order effects had occurred for any of the outcome variables of this study, and no significant gender differences were evident.

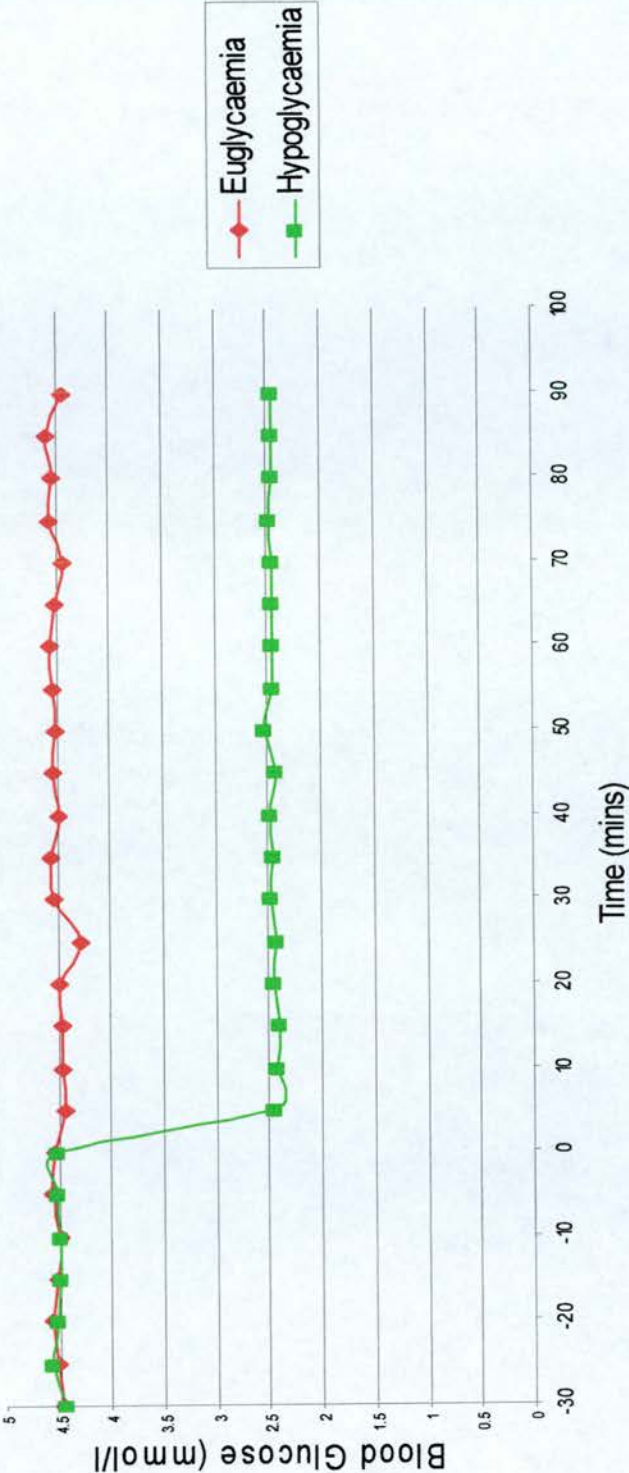
Symptoms

Results of the hypoglycaemia symptom questionnaires confirmed that scores for autonomic ($p<0.0001$), neuroglycopenic ($p<0.0001$) and general malaise ($p=0.05$) symptoms were all increased significantly during hypoglycaemia compared to baseline euglycaemia, and were unchanged during the euglycaemia study condition.

Tests of General Cognitive Function

Considerable differences were observed during hypoglycaemia in the performance of the Trail Making B and Digit Symbol tests. The time taken to complete the Trail Making B test increased significantly from a mean (SD) of 41.5 (7.9) seconds during euglycaemia to 62.6 (11.0) seconds during hypoglycaemia ($p<0.0001$, effect size 0.807). The mean (SD) score of the Digit Symbol test deteriorated from 70.1 (13.0) during euglycaemia to 59.6 (14.4) during hypoglycaemia ($p=0.001$, effect size=0.576).

Figure 7.2: Mean blood glucose profiles during euglycaemia and hypoglycaemia studies.



Tests of Memory

The results of the memory function tests are summarised in Table 7.1.

Immediate Verbal Memory

Acute hypoglycaemia caused a significant deterioration in tests of immediate verbal memory as assessed by the AVLT and Logical Memory tests (Figure 7.3). The mean (SD) immediate AVLT score measured 43.6 (7.0) during euglycaemia and 33.9 (7.7) during hypoglycaemia ($p=0.001$, effect size=0.535). Immediate Logical Memory also demonstrated a significant deterioration during hypoglycaemia, with a mean (SD) of 27.7 (5.9) during euglycaemia, falling to 24.4 (5.0) during hypoglycaemia ($p=0.004$, effect size=0.453).

Immediate Visual Memory

In the Benton Visual Retention Test, the achieved score declined from a mean (SD) of 6.3 (1.7) during euglycaemia to 5.1 (1.6) during hypoglycaemia ($p=0.041$, effect size=0.266) (Figure 7.3). By contrast, the results for the Visual Reproduction test did not demonstrate a significant deterioration (Figure 7.3). The score fell from a mean (SD) of 85.6 (8.3) during euglycaemia to 81.7 (8.0) during hypoglycaemia ($p=0.059$). Despite failing to achieve significance, the effect size was moderate (effect size=0.232).

Delayed Verbal Memory

Both the AVLT Delayed and Logical Memory Delayed tests were affected by hypoglycaemia (Figure 7.4). The scores deteriorated from a mean (SD) of 10.3 (2.1)

and 15.1 (4.0) during euglycaemia to 4.5 (1.6) ($p<0.0001$, effect size=0.873) and 8.1 (2.9) ($p=0.001$, effect size=0.584) during hypoglycaemia respectively.

Delayed Visual Memory

The Visual Reproduction Delayed test demonstrated a significant decrement during hypoglycaemia (Figure 7.4). The mean (SD) score was 21.5 (16.5) during euglycaemia, and 7.6 (5.5) during hypoglycaemia ($p=0.001$, effect size=0.575).

Working Memory

In all tests of working memory the performance deteriorated during hypoglycaemia. In the Digit Span Forwards test, mean (SD) score measured 10.2 (2.4) during euglycaemia, compared to 9.3 (1.7) during hypoglycaemia ($p=0.042$, effect size=0.263). In the Digit Span Backwards, scores deteriorated from a mean (SD) of 8.8 (1.9) during euglycaemia to 6.8 (1.2) during hypoglycaemia ($p<0.0001$, effect size=0.674) (Figure 7.5), and in the Letter/Number Sequencing test total scores were 13.1 (3.1) during euglycaemia and 10.5 (1.8) during hypoglycaemia ($p=0.008$, effect size=0.403) (Figure 7.5). The effects of hypoglycaemia on the Four-term Order and Validation Span tests were also highly significant (Figure 7.5). Four-term order had a mean (SD) score of 11.5 (4.9) during euglycaemia compared to 2.5 (1.5) during hypoglycaemia ($p<0.0001$, effect size 0.8). A large decrement in performance in the Validation Span was also observed during hypoglycaemia; mean (SD) during euglycaemia measured 22.0 (2.3), and during hypoglycaemia measured 13.4 (2.5) ($p<0.0001$, effect size 0.942).

Table 7.1 Results, shown as mean (SD), of tests of memory function during euglycaemia and hypoglycaemia in 16 healthy adults.

Memory system	Subtest	Euglycaemia	Hypoglycaemia	p value	Eta ²
Immediate verbal memory	Immediate Logical Memory	27.7 (5.9)	24.4 (5.0)	.004	.45
	Immediate AVLT	43.6 (7.0)	33.9 (7.7)	.001	.53
Immediate visual memory	Benton Visual Retention test	6.3 (1.7)	5.1 (1.6)	.041	.26
	Visual Reproduction	85.6 (8.3)	81.7 (8.0)	.059	.23
Delayed Memory	Delayed Logical Memory	15.1 (4.0)	8.1 (3.9)	.001	.58
	Percent Retained	88.2 (10.0)	55.56 (19.7)	<.0001	.73
	Delayed AVLT	10.3 (2.1)	4.5 (1.6)	<.0001	.87
	Percent Retained	88.2 (8.9)	50.6 (18.3)	<.0001	.79
Working Memory	Delayed Visual Reproduction	21.5 (16.5)	7.6 (5.5)	.001	.57
	Percent Retained	25.9 (20.3)	10.0 (7.3)	.003	.47
	Validation Span	22.0 (2.3)	13.4 (2.5)	<.0001	.94
	Digit Span Forwards	10.2 (2.4)	9.3 (1.7)	.042	.26
	Digit Span Backwards	8.8 (1.9)	6.8 (1.2)	<.0001	.67
	Letter/Number Sequence	13.1 (3.1)	10.5 (1.8)	.008	.40

AVLT – Auditory Verbal learning Test

SD – Standard Deviation

Figure 7.3: Short-term memory function during euglycaemia and hypoglycaemia in healthy volunteers (n=16). (a) Auditory Verbal Learning Test, (b) Logical Memory, (c) Benton Visual Retention Test, (d) Visual Reproduction. Values shown are mean \pm standard error of the mean (SE).

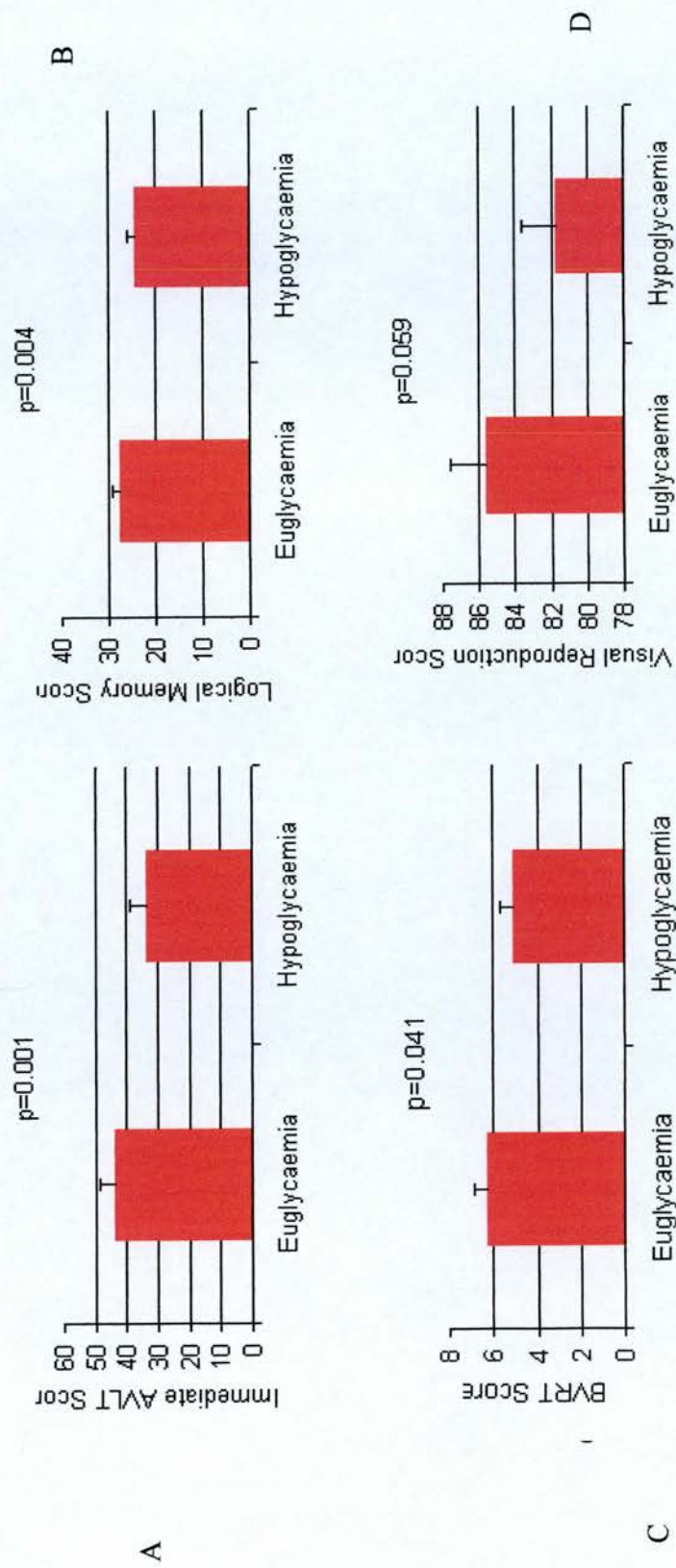
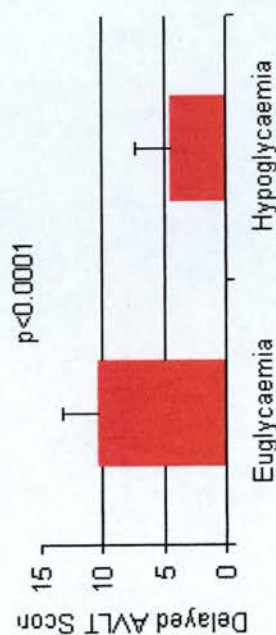
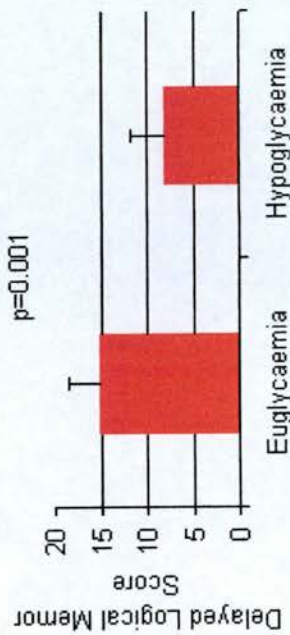


Figure 7.4: Delayed memory function during euglycaemia and hypoglycaemia in healthy volunteers (n=16). (a) Delayed AVLT, (b) Delayed Logical Memory, (c) Delayed Visual Reproduction. Values shown are mean \pm standard error of the mean (SE).

A



B



C

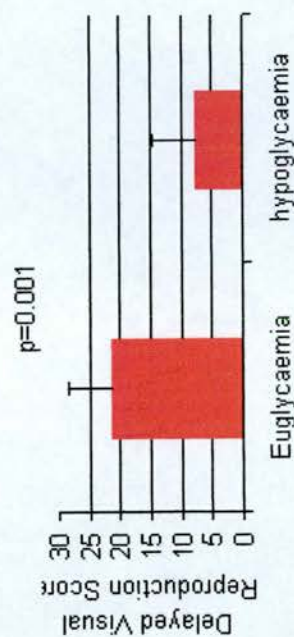
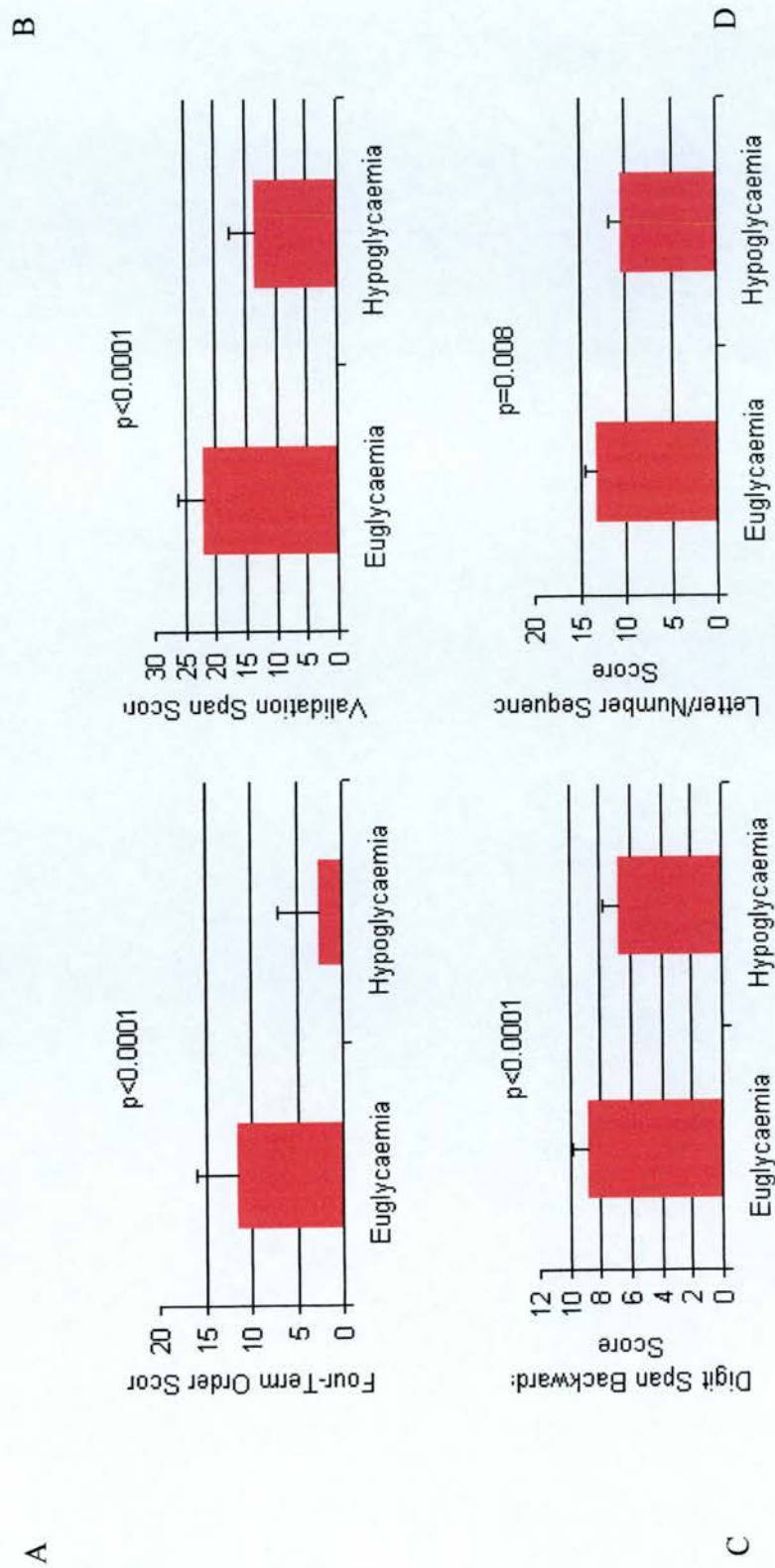


Figure 7.5: Working memory function during euglycaemia and hypoglycaemia in healthy volunteers (n=16). (a) Four-Term Order, (b) Validation Span, (c) Digit Span Backwards, (d) Letter/Number sequence. Values shown are mean \pm standard error of the mean (SE).



DISCUSSION

The present study has demonstrated the profound effect of acute hypoglycaemia on impairing memory function in humans. The results indicate that all memory systems were impaired during hypoglycaemia, with working memory and delayed memory being particularly susceptible. Performance in tests of general cognitive ability, the Trail Making B and Digit Span tests, were also significantly impaired during hypoglycaemia, consistent with the results of previous studies (93,185,217,218,281). This demonstrates that the degree of hypoglycaemia achieved was sufficient to impair other domains of cognitive function simultaneously.

Memory is a crucial domain of cognition, and is fundamental to the successful completion of many everyday tasks. At least three main memory systems – sensory, short-term and long-term memory – are recognised (216). They are discussed in more detail in Chapter 4. Working memory, often regarded as a short-term memory system, is a discrete modality that allows the short-term manipulation of material, as opposed to the storage of material in memory.

Few studies have examined the effects of acute hypoglycaemia on memory function in people with and without diabetes (93,99,152,185,279), and earlier studies have given inconsistent results. In view of the paucity of data on the effects of hypoglycaemia on this important cognitive domain, the present study has attempted a comprehensive examination of the effects of hypoglycaemia on short-term, delayed (longer-term) and working memory.

The present study has shown that short-term memory is significantly disrupted by hypoglycaemia. The effect of hypoglycaemia on short-term verbal memory, assessed by the AVLT and Logical Memory tests, was considerable, with performance in both tests being significantly impaired. More importantly, the extent of the deterioration induced by hypoglycaemia was substantial, as indicated by the large effect size. The extent of deterioration was slightly greater in the AVLT compared to the Logical Memory test. The capacity of short-term memory to retain verbal material depends on how much meaning the information confers to the recipient. A list of random, unrelated words is therefore more difficult to remember than a story in which the words are organised into meaningful sentences with a structured and logical progression. Short-term visual memory was also impaired during hypoglycaemia. The Benton Visual Retention Test score decreased significantly during hypoglycaemia, although this test was impaired to a lesser degree than tests of verbal memory. This may reflect an integral difference in the degree of susceptibility to hypoglycaemia of these two different modalities, or may indicate a relative insensitivity of this specific test in detecting changes in cognitive performance. The immediate Visual Reproduction test was not significantly affected by hypoglycaemia. Although a small decrement was observed in the scores during hypoglycaemia as compared to euglycaemia, this difference was not significant. However, the effect size was modest indicating that, even though the effects of hypoglycaemia on this test did not achieve significance in this study, hypoglycaemia caused an impairment of test performance.

All tests of delayed memory were greatly affected by hypoglycaemia. Performance in the Delayed AVLT and Delayed Logical Memory tests decreased

substantially, with the Delayed AVLT test being more vulnerable to the effects of hypoglycaemia. The effects of hypoglycaemia on delayed visual memory were also considerable. Working memory was very susceptible to the effects of hypoglycaemia, with all tests being affected severely. The Digit Span Forwards test, the least difficult of the working memory tests, was the least affected. Digit Span Backwards, Letter/Number Sequencing, Four-term Order and Validation Span were all more substantially impaired. The impact of hypoglycaemia on performance in these tests was highly significant, and the extent of the decrements, as measured by effect size, was large, especially for the Four-term Order and Validation Span tests.

Much of the knowledge about the brain mechanisms which underlie memory has been derived from animal studies (282) or from studies of humans who have sustained a preceding brain injury (283). Neuroimaging techniques in humans has confirmed the importance of medial temporal lobe structures such as the hippocampus and adjacent parahippocampal regions to memory function in normal individuals (284,285). Injury to the medial temporal lobe yields global amnesia, a profound and pervasive inability to remember new information. The hippocampus is susceptible to a wide variety of toxic insults, including hypoglycaemia.

The brain is susceptible to neuroglycopenia in a rostrocaudal direction with the cerebral cortex and hippocampus being most sensitive and the brainstem and spinal cord being most resistant (35). The brains of human subjects who have suffered an episode of severe hypoglycaemia have been studied and are shown to have areas of cortical necrosis in the frontal lobes and hippocampus, with relative sparing of the hindbrain (91,286). Anecdotal case reports of severe amnesia following an episode of severe

hypoglycaemia have identified a specific structural lesion in the hippocampus in people with insulin-treated diabetes (92,287).

The results of the present study would support the premise that the hippocampus is very vulnerable to the adverse effects of hypoglycaemia. Working memory and delayed memory appeared to be the most vulnerable components of memory to the effects of hypoglycaemia. Many of the tests of working memory also engage other cognitive processes, particularly attention. In the brain, the frontal lobes are important for performance in tasks requiring attention (288,289), and these areas of the brain are also very sensitive to the effects of neuroglycopenia (82,91). A recent study in our own centre has confirmed the detrimental effects of acute hypoglycaemia on attention (290). In the present study, it is possible that the marked disruptive effect of hypoglycaemia on working memory is a combination of the simultaneous adverse effects of hypoglycaemia on impairing memory and attention. The present study also demonstrated that other domains of cognition are impaired during moderately severe hypoglycaemia, and it is feasible that the impairment of memory performance observed in this study reflects a transient global brain dysfunction induced by hypoglycaemia, rather than specific vulnerability of memory processes to hypoglycaemia. The very large effect size associated with the four-term order test test, in particular, may be a consequence of task complexity.

The impact of hypoglycaemia on delayed memory was also profound. It is likely that the normal pathways of hippocampal neural activity that are responsible for the transfer of information from short-term memory to longer-term memory are disrupted by neuroglycopenia, resulting in a propensity for disturbance in tests of delayed memory.

The present study has revealed the adverse effects of hypoglycaemia on impairment of memory in non-diabetic humans and, in particular, has demonstrated the potentially deleterious immediate effects of hypoglycaemia on working and delayed memory. We acknowledge that, with the administration of such an extensive battery of cognitive tests, there is the potential for type 1 statistical error, and ideally this should have been excluded by the use of bonferroni statistical analysis. It is not known whether the effect of hypoglycaemia on memory functions is sustained or protracted, and this requires further investigation. Many tasks of everyday life engage memory processes, especially working memory. For example, working memory is used to remember whether traffic is proceeding along a street after looking to either side before crossing, taking rapid decisions while driving or operating machinery or computer equipment, and is fundamental to following instructions or reading a map. It is difficult to quantify the extent to which memory impairment during acute hypoglycaemia is manifest in everyday life. There is considerable inter-individual variability of susceptibility to the adverse cognitive effects of acute hypoglycaemia and it is probable that some individuals are more profoundly affected than others. People with insulin-treated diabetes are frequently exposed to hypoglycaemia as a result of the limitations of their insulin therapy. It is probable that these individuals will be subject to a similar impairment of memory functions during hypoglycaemia, which could have important practical implications for daily activities and working ability. The detrimental effect of hypoglycaemia on delayed memory also suggests that the ability to learn during hypoglycaemia could be substantially impaired, and could influence academic performance in examinations.

Chapter 8

Short-Term, Delayed and Working Memory are Impaired During Hypoglycaemia in People with Type 1 Diabetes

INTRODUCTION

Hypoglycaemia is a common side-effect of treatment with insulin in people with diabetes (1,33). Acute neuroglycopenia causes a rapid deterioration in cognitive function in humans with, and without, diabetes (235). In general, tests that involve attention, concentration, psychomotor skill, the accessing of long term memory and the ability to ignore distracting information, deteriorate when arterial blood glucose declines below about 3.0 mmol/l (6,82,152,185,196,278).

Memory is one of the most important cognitive domains with respect to everyday function and is the process of storing, encoding and retrieving information. However, few studies have examined the effects of acute hypoglycaemia on memory function, other than by including a heterogeneous assortment of memory measures as part of a larger battery of cognitive tests. In some studies, memory functions were impaired during acute hypoglycaemia (6,82,99,185,279), whereas in others, memory was apparently unaffected (152,239,280). Variability of results may relate to methodological differences. Many studies are limited by small sample size, application of a limited selection of tests, and inadequate ascertainment of biomedical and psychosocial variables that could affect the results. The method of induction of hypoglycaemia has also varied between studies, with differing methods of blood sampling (e.g. arterialised or venous blood). A common methodological error has been the induction of a stepwise decline in blood glucose with estimation of cognitive function during the final 30 minutes of each blood glucose plateau, thus confounding glucose level with practise effects.

In our laboratory a comprehensive examination of the effects of moderate acute hypoglycaemia on memory performance in healthy non-diabetic humans demonstrated global impairment of all memory systems, with working memory and delayed memory being most susceptible to the effects of neuroglycopenia (292). The present study was designed to investigate the effects of experimentally-induced hypoglycaemia on verbal and non-verbal tests of short-term, delayed and working memory in adults with type 1 diabetes, many of whom are frequently exposed to mild (self-treated) hypoglycaemia.

METHODS

Subjects

Sixteen (9 male) adults with type 1 diabetes were studied. Subjects were recruited from the diabetes out-patient clinics at the Royal Infirmary of Edinburgh. Their median (range) age was 28.5 (20.0-38.2) years, with a Body Mass Index of 23.9 (20.1-24.8) kg/m², duration of diabetes of 4.5 (1.2-8.4) years, HbA1c 8.2 (6.9-8.7) %, and insulin dose of 0.65 (0.25-1.1) u/kg. HbA1c was measured by High Performance Liquid Chromatography (Variant II Haemoglobin Testing System, Biorad Diagnostics Group, Hercules, California, USA). None of the participants had a history of hypertension or chronic disease, previous head injury, seizure, blackouts, alcohol or drug abuse, or psychiatric illness. They had no intercurrent illness and were not taking any regular medication (with the exception of insulin and the oral contraceptive pill). Subjects were excluded if they had any evidence of microvascular disease. The presence of retinopathy was ascertained by ophthalmoscopy, neuropathy by clinical examination, and nephropathy was defined by the presence of microalbuminuria. Subjects were excluded if they had a history of impaired awareness of hypoglycaemia, or had suffered an episode of hypoglycaemia in the 48 hours preceding the study. Ethical permission for the study was approved by the Lothian Research Ethics Committee. All subjects gave written informed consent for participation in the study.

Study Design

Each subject participated in two laboratory sessions that were separated by at least two weeks (Figure 8.1). The studies were conducted in the Department of Diabetes at the Royal Infirmary of Edinburgh. A modified hyperinsulinaemic glucose clamp (238) was used to maintain the blood glucose at a predetermined level. In the *euglycaemia* study, the arterialised blood glucose concentration was maintained at 4.5 mmol/l and hypoglycaemia was not induced. In the *hypoglycaemia* study, the glucose concentration was lowered to 2.5 mmol/l. The subjects were not informed which experimental arm of the study was being undertaken on each occasion and the two experimental sessions were performed in a randomized and counterbalanced fashion.

Procedure

The hyperinsulinaemic glucose clamp procedure (238), as discussed in chapter 6, was used to induce hypoglycaemia.

Cognitive Function Tests

Tests of immediate and delayed verbal memory, immediate and delayed visual memory, and tests of working memory were administered during the study conditions. In addition to the memory tests, the Trail Making B and Digit Symbol tests were also administered. These are validated tests of general cognitive ability and were used to confirm the effect of hypoglycaemia on cognitive function, as has been shown in previous studies (239,279,280,291).

Symptoms of Hypoglycaemia

The Edinburgh Hypoglycaemia Scale (276) was used to document the common symptoms of hypoglycaemia experienced by the subjects during the two studies.

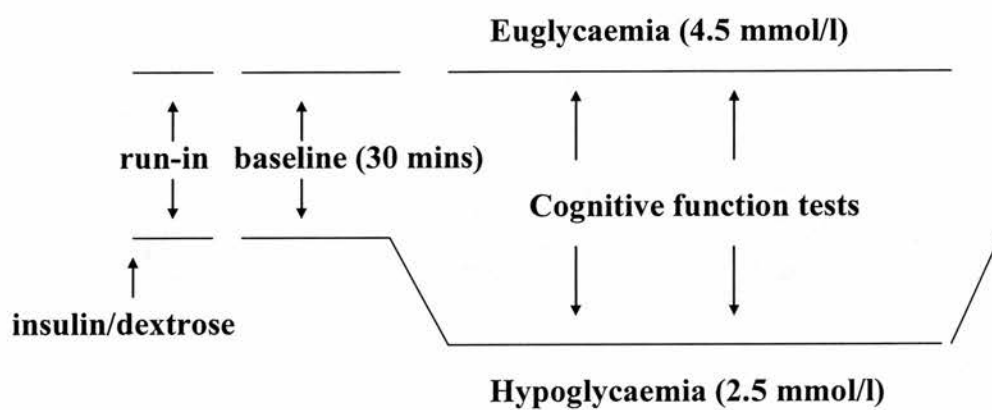


Figure 8.1: Study design

RESULTS

A stable blood glucose plateau was achieved during each study condition (Figure 8.2). The mean (SD) arterialised blood glucose concentration during the euglycaemia condition was 4.55 (0.18) mmol/l, and during the hypoglycaemia condition was 2.51 (0.08) mmol/l. Statistical analysis revealed that no significant order effects had occurred for any of the outcome variables of this study, and no significant gender differences were evident.

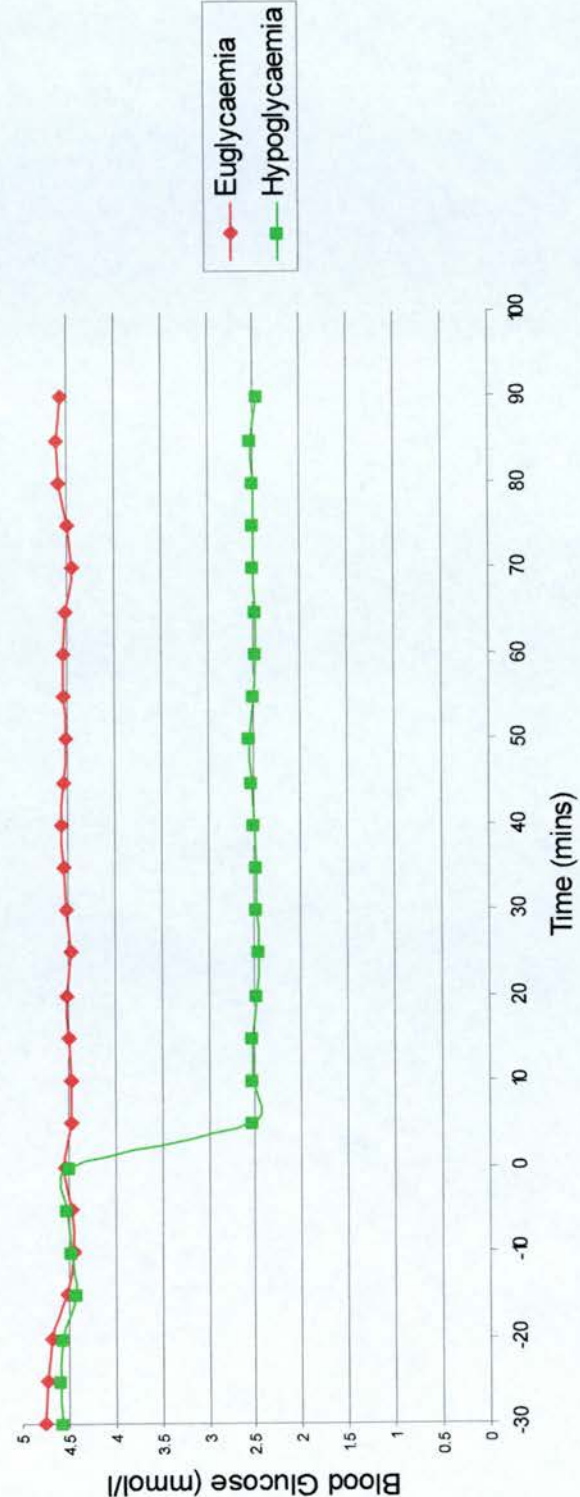
Symptoms

Results of the hypoglycaemia symptom questionnaires confirmed that scores for autonomic ($p<0.0001$), neuroglycopenic ($p=0.001$) and general malaise ($p=0.008$) symptoms were all significantly elevated during hypoglycaemia compared to baseline euglycaemia, and were unchanged from baseline levels during the euglycaemia study condition.

Digit Symbol and Trail Making B

During hypoglycaemia, the time taken to complete the Trail Making B test increased significantly from a mean (SD) of 33.7 (7.7) seconds during euglycaemia to 54.0 (10.7) seconds during hypoglycaemia ($p<0.0001$, $\eta^2=0.68$). The mean (SD) score of the Digit Symbol test declined from 73.5 (11.2) during euglycaemia to 62.9 (16.9) during hypoglycaemia ($p=0.001$, $\eta^2=0.57$).

Figure 8.2: Mean blood glucose profiles during euglycaemia and hypoglycaemia studies.



Tests of Memory

The results of the memory function tests are summarised in Table 8.1.

Immediate Verbal Memory

Acute hypoglycaemia caused a significant deterioration in tests of immediate verbal memory as assessed by the AVLT ($p=0.002$, $\eta^2=0.49$) and Logical Memory tests ($p=0.008$, $\eta^2=0.41$) (Figure 8.3).

Immediate Visual Memory

The Benton Visual Retention Test score declined during hypoglycaemia ($p=0.007$, $\eta^2=0.42$). By contrast, the score for the Visual Reproduction test did not fall significantly ($p=0.093$) (Figure 8.3).

Delayed Verbal Memory

Both the AVLT Delayed ($p<0.0001$, $\eta^2=0.68$. Percent retained: $p<0.0001$, $\eta^2=0.78$) and Logical Memory Delayed ($p<0.0001$, $\eta^2=0.83$. Percent retained: $p<0.0001$, $\eta^2=0.73$) scores were significantly worse during hypoglycaemia (Figure 8.4).

Delayed Visual Memory

During hypoglycaemia a significant decrement was observed in the Visual Reproduction Delayed test ($p=0.002$, $\eta^2=0.52$. Percent retained: $p=0.004$, $\eta^2=0.46$) (Figure 8.4).

Working Memory

Performance in the working memory tests is shown in Figure 8.5. Digit Span Backwards ($p=0.02$, $\eta^2=0.33$), Letter/Number Sequencing ($p=0.001$, $\eta^2=0.57$) and Validation Span ($p<0.0001$, $\eta^2=0.92$) all demonstrated a significant decrement during hypoglycaemia. The Validation Span test declined from a mean (SD) score of 20.7 (2.3) during euglycaemia to 14.9 (2.2) during hypoglycaemia. However, performance in the Digit Span Forwards test was not significantly affected ($p=0.06$, $\eta^2=0.23$).

Table 8.1: Results, shown as mean (SD), of tests of memory function during euglycaemia and hypoglycaemia in 16 adults with type 1 diabetes.

Memory system	Subtest	Euglycaemia	Hypoglycaemia	p value	Eta ²
Immediate verbal memory	Immediate Logical Memory	26.8 (4.8)	21.3 (6.9)	.008	.41
	Immediate AVLT	39.4 (7.7)	34.0 (5.1)	.002	.49
Immediate visual memory	Benton Visual Retention test	6.5 (1.7)	4.7 (1.5)	.007	.42
	Visual Reproduction	81.3 (7.2)	78.2 (9.5)	.093	.19
Delayed Memory	Delayed Logical Memory	13.6 (2.1)	6.1 (3.7)	< .0001	.83
	Percent Retained	78.4 (6.0)	47.0 (21.3)	< .0001	.73
	Delayed AVLT	9.1 (1.9)	5.3 (1.9)	< .0001	.68
	Percent Retained	79.1 (6.9)	47.8 (16.0)	< .0001	.78
Working Memory	Delayed Visual Reproduction	15.9 (8.7)	7.1 (7.3)	.002	.52
	Percent Retained	18.6 (9.8)	9.1 (9.0)	.004	.46
	Validation Span	20.7 (2.3)	14.9 (2.2)	< .0001	.92
	Digit Span Forwards	9.56 (2.4)	8.6 (1.4)	.06	.23
	Digit Span Backwards	8.3 (1.9)	7.2 (1.2)	.02	.33
	Letter/Number Sequence	11.8 (1.9)	9.7 (1.6)	.001	.57

AVLT – Auditory Verbal learning Test SD – Standard Deviation

Figure 8.3: Short-term memory function during euglycaemia and hypoglycaemia in people with Type 1 diabetes (n=16). (a) Auditory Verbal Learning Test, (b) Logical Memory, (c) Benton Visual Retention Test, (d) Visual Reproduction. Values shown are mean \pm standard error of the mean (SE).

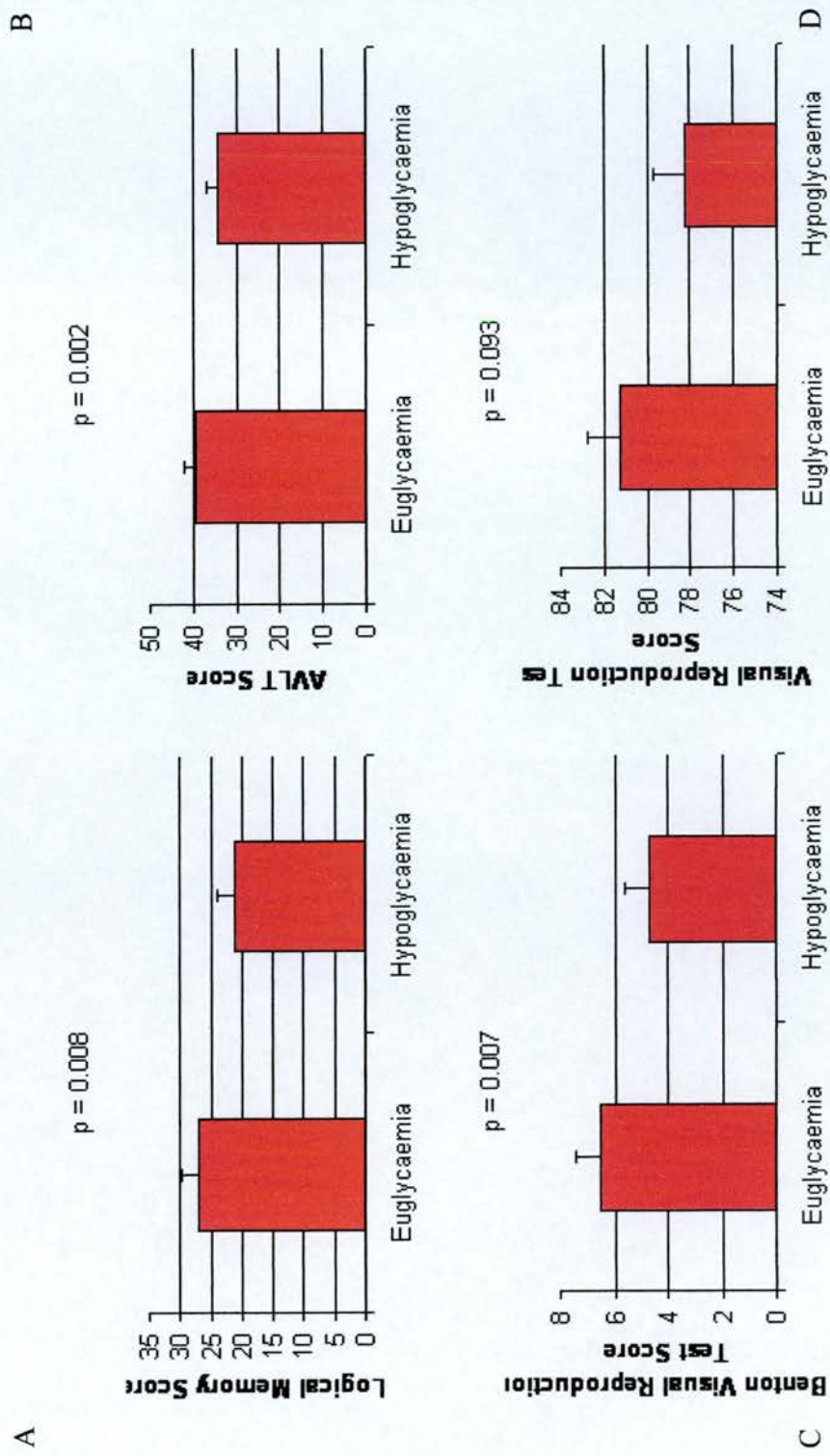


Figure 8.4: Delayed memory function during euglycaemia and hypoglycaemia in people with Type 1 diabetes (n=16). (a) Delayed AVLT, (b) Delayed Logical Memory, (c) Delayed Visual Reproduction. Values shown are mean \pm standard error of the mean (SE).

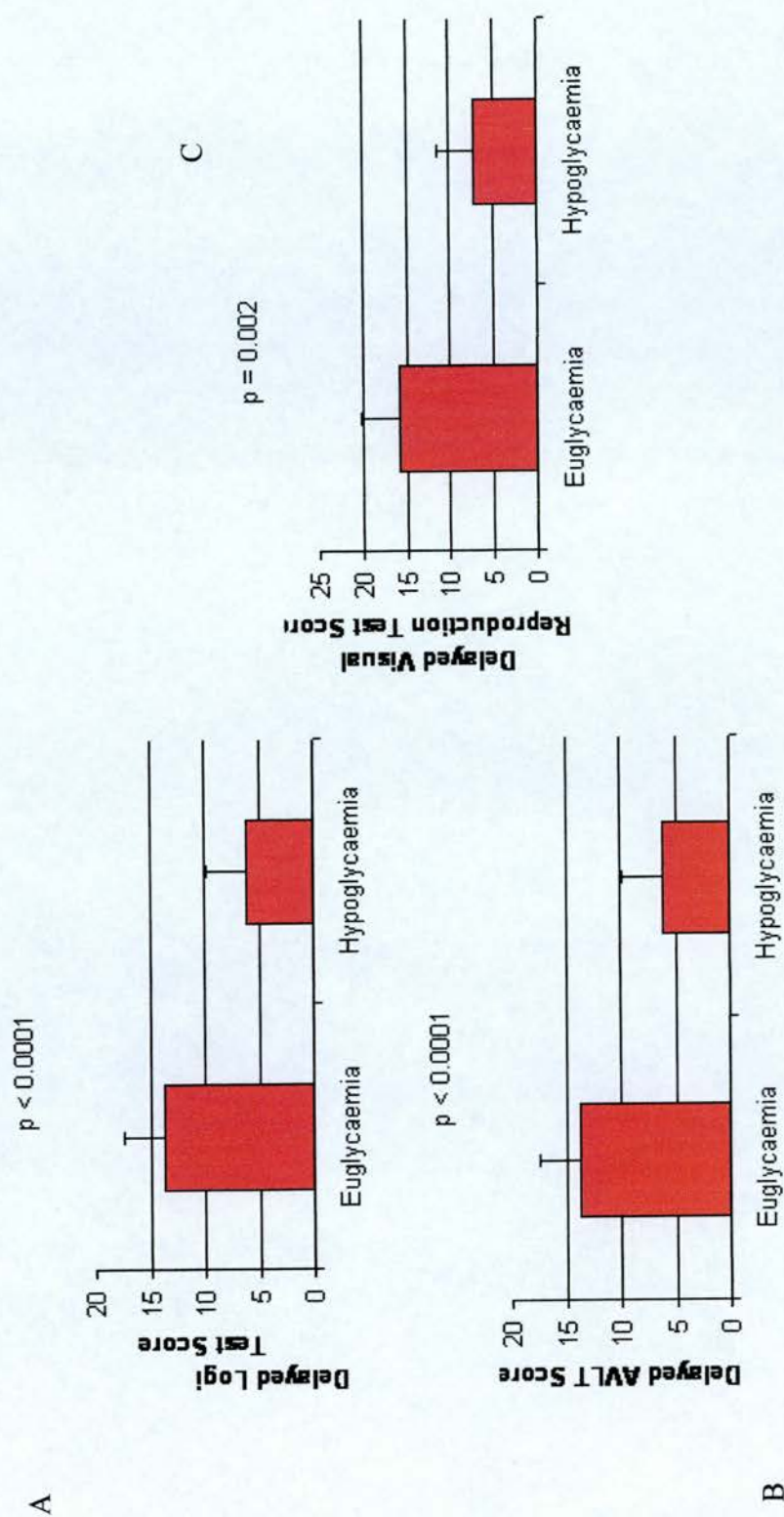
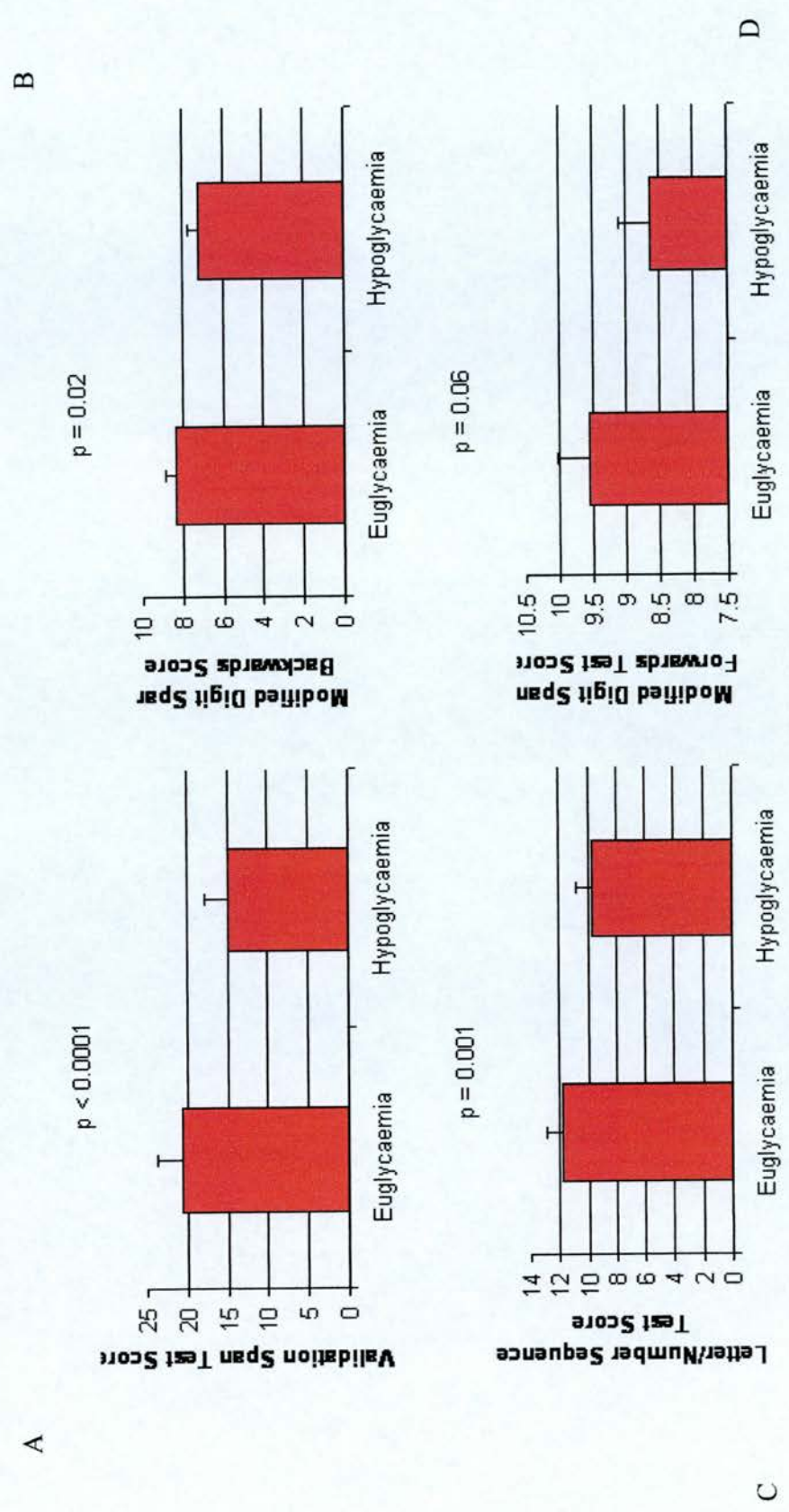


Figure 8.5: Working memory function during euglycaemia and hypoglycaemia in people with Type 1 diabetes (n=16). (a) Validation Span, (b) Digit Span Backwards, (c) Letter/Number sequence, (d) Digit Span Forwards. Values shown are mean \pm standard error of the mean (SE).



DISCUSSION

Few previous studies have made a detailed examination of the effects of acute hypoglycaemia on memory function in humans, with and without diabetes (99,152,185,239,279), and earlier studies have given inconsistent results. Using an identical study design, we have studied the effects of hypoglycaemia in young, healthy, non-diabetic volunteers and demonstrated a profound impairment in memory function at a blood glucose level that was similar to the present study (2.5 mmol/l) (292). In view of the frequency with which mild hypoglycaemia is experienced by people with insulin-treated diabetes, the present study has examined the effects of acute hypoglycaemia on a wide range of memory functions in a group of adults with type 1 diabetes who had no evidence of significant vascular complications.

The present study has demonstrated that in adults with type 1 diabetes acute moderate hypoglycaemia caused a marked deterioration in performance in tests of short-term, delayed and working memory, for both verbal and non-verbal material, with working memory and delayed memory being affected most strongly (according to effect size analysis). In addition, performance in the Trail Making B and Digit Symbol tests was impaired significantly during hypoglycaemia. This is consistent with previous observations (185,217,218,279,281), and confirms that the degree of hypoglycaemia achieved was sufficient to affect other domains of cognitive function.

Short-term memory was significantly disrupted by hypoglycaemia in the present study. The effect of hypoglycaemia on short-term verbal memory, assessed by the AVLT and Logical Memory tests, and short-term visual memory, assessed by the

Benton Visual Retention Test, was profound, with performance in the three tests being significantly impaired. Earlier studies have demonstrated decrements in short-term, verbal memory during hypoglycaemia (99,185). In the present study, the magnitude of the impairment induced by hypoglycaemia was substantial, as indicated by the large effect size. However, the immediate Visual Reproduction test was not significantly affected by hypoglycaemia. This appeared to be a manifestation of a 'ceiling-effect' (i.e. most subjects obtained near-to-perfect scores), indicating a lack of sensitivity of this test to the stress of hypoglycaemia.

A significant deterioration in performance in tests of delayed verbal memory has been shown during hypoglycaemia in previous human studies of non-diabetic (6) and diabetic subjects (162). In the present study, all tests of delayed memory were markedly affected by hypoglycaemia. Performance in the Delayed AVLT and Delayed Logical Memory tests deteriorated significantly during hypoglycaemia, and the effects of hypoglycaemia on delayed visual memory were also considerable. Analysis of the 'immediate' and 'delayed' Logical Memory, AVLT and Visual Reproduction test scores allowed calculation of the percentage of information that was learned and retained during the study. This revealed that, for tests of both verbal and visual memory, not only was less information recalled immediately after having studied the information during hypoglycaemia as compared to euglycaemia but that, over time, subjects forgot more of what they had recalled immediately during hypoglycaemia, than during the euglycaemic state.

Working memory was very susceptible to the effects of hypoglycaemia. The Digit Span Forwards test, the least difficult of the working memory tests, was the least

affected and was not significantly impaired during hypoglycaemia. A small decrement was observed in the scores during hypoglycaemia as compared to euglycaemia and the effect size was modest, indicating that, although the effects of hypoglycaemia on this test did not achieve significance in this study, hypoglycaemia did impair test performance. Digit Span Backwards, Letter/Number Sequencing and Validation Span were all more substantially impaired. The impact of hypoglycaemia on performance in these tests was highly significant, and the extent of the decrements, as measured by the effect size, was large.

Functional neuroimaging studies in humans have confirmed that medial temporal lobe structures such as the hippocampus and adjacent parahippocampal regions are the principal structures involved with memory performance (284,285). The hippocampus is susceptible to a variety of toxic insults, including heavy metals (289), hypoxia (290), and drugs (295,296). There is some evidence that this particular area of the brain is also preferentially vulnerable to the adverse effects of hypoglycaemia. Neuropathological observations have indicated that the brain is sensitive to neuroglycopenia in a rostrocaudal direction with the cerebral cortex and hippocampus being most susceptible and the brainstem and spinal cord being most resistant (91). The brains of human subjects who have suffered an episode of severe hypoglycaemia have been studied and are shown to have areas of cortical necrosis in the frontal lobes and hippocampus, with relative sparing of the hindbrain (91,286). Anecdotal case reports of severe amnesia following an episode of severe hypoglycaemia have identified a specific structural lesion in the hippocampus in people with insulin-treated diabetes (92,287).

The results of the present study have clearly demonstrated the short-term detrimental effects of acute hypoglycaemia on memory and are consistent with these earlier observations. We acknowledge that, with the administration of such an extensive battery of cognitive tests, there is the potential for type 1 statistical error, and ideally this should have been excluded using bonferroni statistical analysis. Working memory and delayed memory appeared to be the most vulnerable components of memory to the effects of hypoglycaemia. Many of the tests of working memory also engage other cognitive processes, particularly attention. In the brain, the frontal lobes are important for performance in tasks requiring attention (288,289). These areas of the brain are also very sensitive to the effects of neuroglycopenia (91), and a different study in our own centre has confirmed the detrimental effects of acute hypoglycaemia on attention (290). In the present study, it is possible that the marked disruptive effect of hypoglycaemia on working memory is a combination of the simultaneous adverse effects of hypoglycaemia on memory and attention. The prefrontal lobe and medial temporal lobe structures are important for performance in tasks of working memory (297,298).

In animals, working memory tasks have been shown to exert high demands on brain extracellular glucose (299). In the brain, glucose is transported across the cell membranes by facilitated diffusion, mediated by the glucose transport proteins GLUT1 and GLUT3 (300). It is possible that the vulnerability of working memory to the effects of hypoglycaemia is a consequence of the interaction between local brain demands and the regional distribution of GLUT1 and GLUT3 receptors. The profound impact of hypoglycaemia on delayed memory is likely to be due to the disruption, by neuroglycopenia, of the normal pathways of hippocampal neural activity that are

responsible for the transfer of information from short-term memory to longer-term memory.

The adverse effects of hypoglycaemia on impairment of memory in adults with type 1 diabetes that have been demonstrated in the present study are very similar to the effects of hypoglycaemia on memory function in non-diabetic humans that we have shown previously (292). It is not known whether the effect of hypoglycaemia on memory is sustained or protracted, and this will require further investigation. Many aspects of cognitive function have been observed to return to normal within two hours after euglycaemia is restored (95,100,301), and this is presumably similar for memory function. The present study also demonstrated that other domains of cognition are impaired during moderately severe hypoglycaemia, and it is feasible that the impairment of memory performance observed in this study reflects a transient global brain dysfunction induced by hypoglycaemia, rather than specific vulnerability of memory processes to hypoglycaemia. The very large effect size associated with the validation span test, in particular, may be a consequence of task complexity.

The effect of memory dysfunction during acute hypoglycaemia on performance in activities during everyday life is difficult to quantify. It is known that there is considerable inter-individual variability of susceptibility to the adverse cognitive effects of acute hypoglycaemia and it is probable that some individuals are more profoundly affected than others. Many tasks of everyday life engage memory processes, especially working memory. For example, before crossing a street, working memory is used by a pedestrian to remember whether traffic is approaching after looking each way, working memory is essential when taking rapid decisions while driving, and is fundamental to

following directions such as reading a map. It is also of critical importance in the work environment and is used continuously when operating machinery or computer equipment. People with insulin-treated diabetes are frequently exposed to varying degrees of hypoglycaemia, and current therapeutic policies that strive to achieve strict glycaemic control may promote more frequent hypoglycaemic events. The results of the present study demonstrate that many people with type 1 diabetes are subject to impairment of memory functions during hypoglycaemia in their everyday lives, which may have important practical implications for daily activities including effective working ability and driving performance (76).

Chapter 9

Working Memory Performance is Particularly Susceptible to the Adverse Effects of Acute Hypoglycaemia

The effects of acute hypoglycaemia on the specific domain of working memory, in both diabetic and non-diabetic subjects, was particularly marked. Working memory is the mental capacity simultaneously to store and manipulate information (224). As has been discussed, glucose is the brain's principal fuel and mental function deteriorates when arterial glucose falls below 3 mmol/l. Tasks that are complex and performed under time pressure are particularly affected, the usual finding being a modest reduction in performance (175). The effect of acute hypoglycaemia on working memory has not been studied previously.

Memory performance was studied in 16 (9 men) healthy, non-diabetic humans, aged 26 and 34 years, under conditions of euglycaemia (4.5 mmol/l) and hypoglycaemia (2.5 mmol/l), using the hyperinsulinaemic glucose clamp technique to achieve precise control of arterialised plasma glucose (238).

The 'Four-Term Order' working memory test is from Kyllonen's well-validated cognitive battery (302). Subjects listen to three rules read aloud by the experimenter and then choose which of eight response options correctly accords with the rules. Eight response alternatives are then displayed on a card and the subject is required to identify the correct sequence as determined by the preceding commands. Twenty four different items were used in each condition.

Performance on the Four-Term Series task fell from 11.5 (4.9) during euglycaemia to 2.5 (1.5) during hypoglycaemia ($p < .0001$). A range of individual differences during euglycaemia, from chance to near-perfect performance, was reduced to near-uniform chance responding during hypoglycaemia (Figure 9.1). Figure 9.1 also demonstrates the performance of the same subjects under the same conditions in the digit symbol task, a test that has been used extensively under the conditions of acute hypoglycaemia. Performance

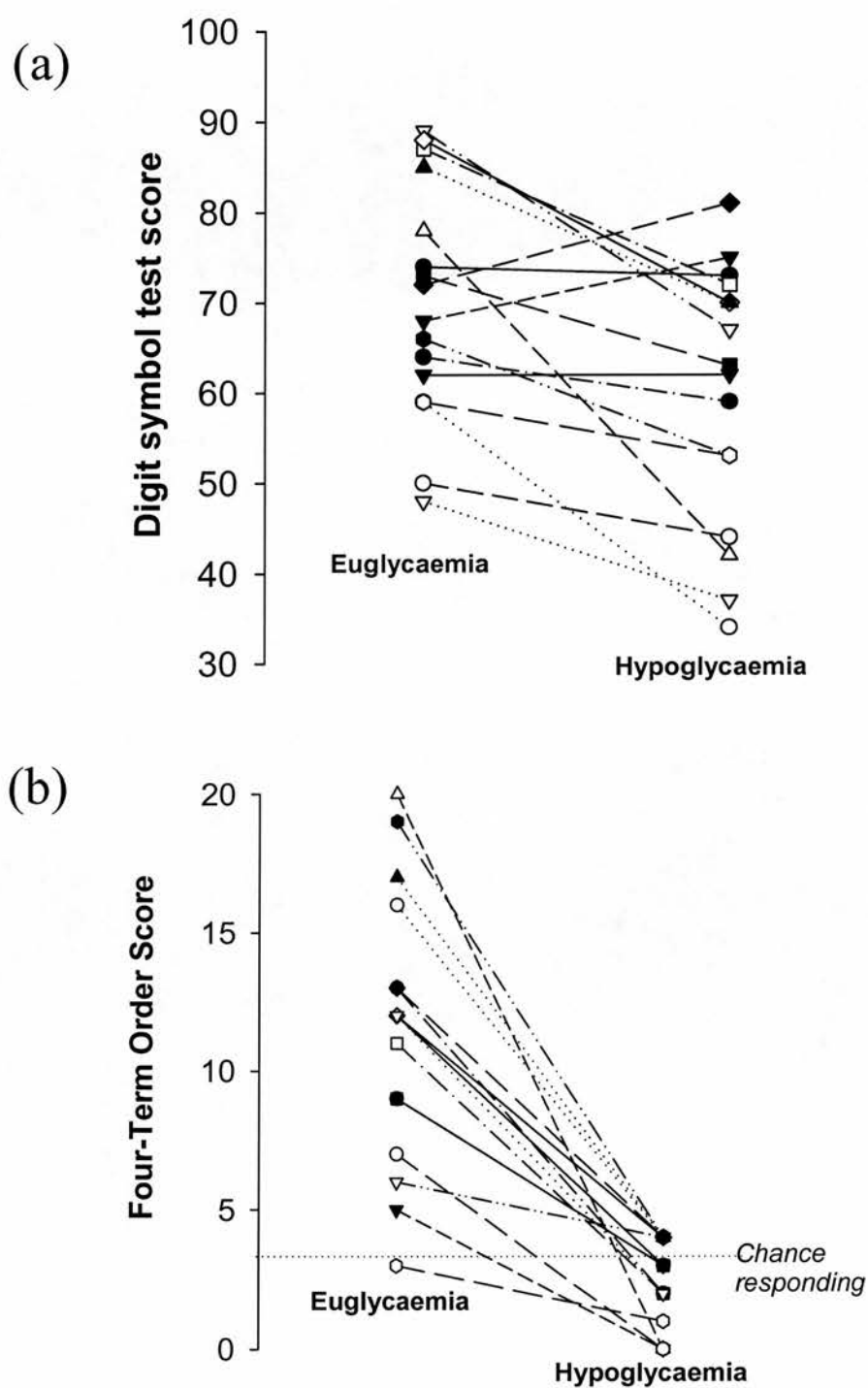
in this particular test was, as expected, significantly poorer during moderate hypoglycaemia (a mean (SD) of 70.1 (13.0) during euglycaemia fell to 59.6 (14.4) during hypoglycaemia) but the range of impairment was substantially greater amongst individuals, with considerable individual variation in response, and considerable overlap in scores between the two conditions.

Unlike other mental functions, working memory performance, as indexed by the Four Term Order task, was reduced to chance levels. Performance in this working memory task was almost obliterated during moderate hypoglycaemia in young, healthy humans. Working memory is a pillar of human mental performance, almost indistinguishable from reasoning and general intelligence (227,302) and such detrimental effects on this particular cognitive domain may, therefore, have substantial detrimental effects on the everyday lives of people with diabetes who are frequently exposed to similar levels of hypoglycaemia. Animal studies suggest that working memory makes high demands on brain extracellular glucose, specifically in the hippocampus (299), and it appears that the hippocampus and other medial temporal lobe structures and the frontal lobes are especially sensitive to the effects of neuroglycopenia (286). The dorsolateral prefrontal cortex is frequently activated during memory and reasoning tasks that involve the evaluation of externally generated information (303,304). A possible basis for the vulnerability of working memory to the effects of hypoglycaemia, therefore, is the interaction of local brain energy demands and the regional density of the cerebral glucose transporters GLUT1 and GLUT3 (300).

The degree of hypoglycaemia induced in this study, which occurs commonly in people with type 1 (insulin-dependent) diabetes, does not affect conscious level. Although

performance in many mental tasks show some deterioration in this state, such a deleterious effect on such a fundamental cognitive domain has not been observed previously.

Figure 9.1: The effect of hypoglycaemia on individual non-diabetic subjects' (a) digit symbol test scores, and (b) working memory test scores (Four-Term Order test) (N = 16).



PART IV

HYPERGLYCAEMIA AND COGNITIVE FUNCTION

Chapter 10

Acute Hyperglycaemia Alters Mood State and Impairs Cognitive Performance in People with Type 2 Diabetes

INTRODUCTION

Diabetes is frequently associated with acute metabolic disturbances and fluctuations in glycaemic control. Hyperglycaemia commonly occurs as a consequence of the relative or absolute insulin deficiency that is intrinsic to diabetes, and hypoglycaemia is a common side effect of the treatment of diabetes with insulin, and to a lesser extent with sulphonylureas (1). The brain is vitally dependent on glucose as a source of fuel, and disturbance of blood glucose concentration can impinge on cerebral function. Many studies have demonstrated the deleterious effects of acute *hypoglycaemia* on cognitive function and on mood states, both in non-diabetic subjects and in people with type 1 and type 2 diabetes (175,235). In contrast, considerably less information is available on the effects of acute *hyperglycaemia* on cognition. However, anecdotal descriptions by patients with either type 1 or type 2 diabetes suggest that altered mood (such as increased irritability and feelings of diminished well-being) and difficulties with rapid cerebation are common when blood glucose is elevated.

Published data on the effects of hyperglycaemia on cognitive function reveals conflicting results. One study (239) demonstrated impaired performance in verbal cognitive tasks in 12 people with type 1 diabetes during acute hyperglycaemia (blood glucose 16.6 mmol/l) and a further study in 12 children, mean age 12.4 years (240) showed a reduction in performance IQ under conditions of controlled hyperglycaemia (20 – 30mmol/l).

In contrast to this, other studies do not support these findings. A study of adolescents with type 1 diabetes did not show any effect of acute hyperglycaemia on

cognitive function (241), and Hoffman *et al* (278) also failed to demonstrate any significant effect of acute hyperglycaemia on cognitive function in 18 adults with type 1 diabetes. Two further studies examined the effects of acute hyperglycaemia on cognitive function (99) and mood (242) in a cohort of 42 adult patients with type 1 diabetes. Neither cognitive function, nor self-reported mood were significantly affected. However, the study cohort had poor metabolic control, with a mean HbA1c of 10.1%. There may be potential for cerebral adaptation in response to a high prevailing blood glucose concentrations.

A further study in non-diabetic volunteers utilised functional magnetic resonance imaging to determine the effects of acute hyperglycaemia on activation of the occipital cortex in response to a visual stimulus (305). The study demonstrated that acute hyperglycaemia did not have a substantial effect on the size or intensity of visual cortical activation in response to visual stimuli.

All previous studies on the effects of acute hyperglycaemia on cognitive function have been in people with type 1 diabetes. To our knowledge, there are no studies on the effects of acute hyperglycaemia on cognitive and non-cognitive function in people with type 2 diabetes. There is increasing evidence that people with type 2 diabetes are at an increased risk of cognitive dysfunction, especially of verbal memory (243). It is probable that this dysfunction is a consequence of synergistic interaction between metabolic derangements associated with diabetes and the structural and functional changes that occur within the central nervous system as part of the normal ageing process. Therefore, people with type 2 diabetes may be more susceptible to cognitive dysfunction during acute glycaemic changes.

The present study aimed to examine the effects of acute hyperglycaemia on cognitive function and mood state in people with type 2 diabetes.

METHODS

Subjects

Twenty (12 male) adults with type 2 diabetes were studied. Subjects were recruited from the diabetes out-patient clinics at the Royal Infirmary of Edinburgh. Their median (range) age was 61.5 (53.1-72.0) years, with a Body Mass Index of 29.8 (22.0-34.6) kg/m², duration of diabetes of 5.9 (2.8-11.2) years, and HbA1c 7.5 (6.7-8.4) %. HbA1c was measured by High Performance Liquid Chromatography (Variant II Haemoglobin Testing System, Biorad Diagnostics Group, Hercules, California, USA) (non-diabetic reference range 4.3-6.5%). Three of the subjects were taking insulin alone for the treatment of their diabetes, five subjects were taking a single oral hypoglycaemic agent (OHA), nine were taking two OHAs, and three subjects were taking a combination of insulin and an OHA. None of the participants had a history of chronic disease, previous head injury, seizure, blackouts, alcohol or drug abuse, or psychiatric illness. Subjects were excluded if they had any evidence of microvascular disease, with the exception of background retinopathy. The presence of retinopathy was ascertained by ophthalmoscopy, neuropathy by clinical examination, and nephropathy was defined by the presence of microalbuminuria. Ethical permission for the study was approved by the Lothian Research Ethics Committee. All subjects gave written informed consent for participation in the study.

Study Design

Each subject participated in two laboratory sessions that were separated by at least two weeks (Figure 10.1). The studies were conducted in the Department of Diabetes at the Royal Infirmary of Edinburgh. A modified hyperinsulinaemic glucose clamp (238) was used to maintain the blood glucose at a predetermined level. In the *euglycaemia* study, the arterialized blood glucose concentration was maintained at 4.5 mmol/l. In the *hyperglycaemia* study, the glucose concentration was raised to 16.5 mmol/l. The subjects were not informed which experimental arm of the study was being undertaken on each occasion and the two experimental sessions were performed in a randomised and counterbalanced fashion.

Procedure

A hyperinsulinaemic glucose clamp was used to induce hyperglycaemia or euglycaemia (238).

Cognitive Function Tests

Validated tests of information processing, tests of memory and tests of attention were administered during each study condition.

Test of information processing included the Trail Making B test, the Digit Symbol Test and the simple and four-choice reaction time tests. In this study, a computerised version of the test on a hand-held computer was used (306).

Tests of memory included verbal memory tests (Auditory Verbal Learning Test (AVLT) – immediate and delayed, Logical Memory Test – immediate and delayed), visual memory tests (Visual Reproduction – immediate and delayed, Benton Visual Retention Test (BVRT)) and working memory tests (Digit Span – Forwards and Backwards, Letter/Number Sequencing)

The Test of Everyday Attention (TEA) battery (274) was used to measure attention.

The UWIST Mood Questionnaire (275) was used to assess changes in mood.

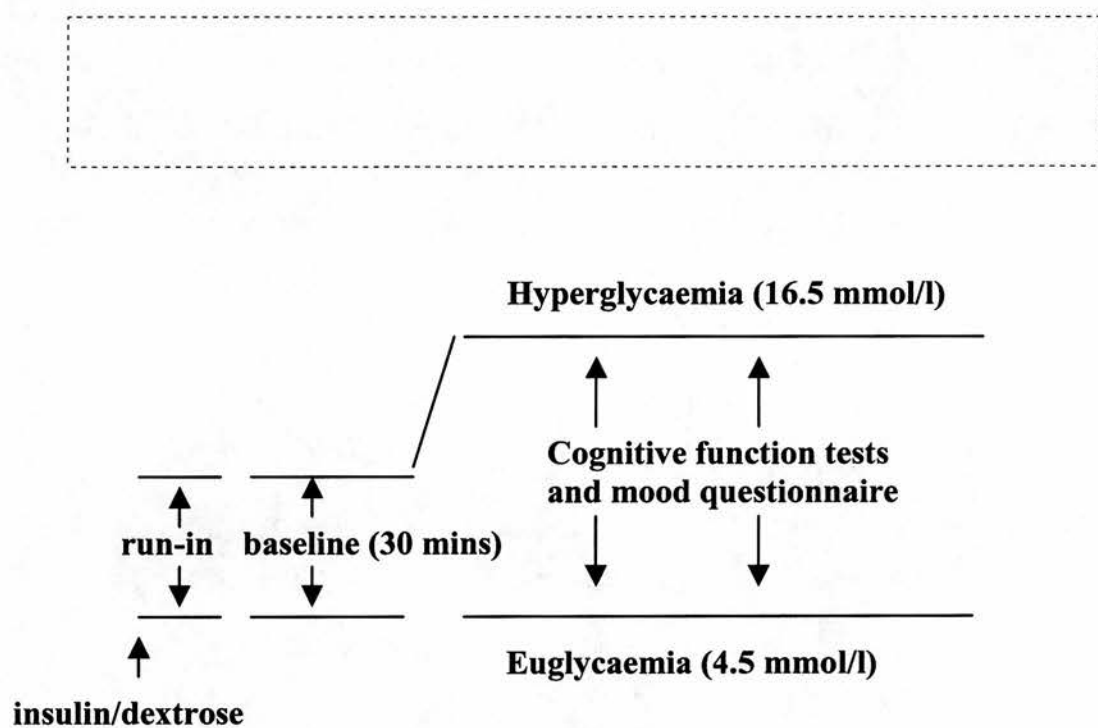


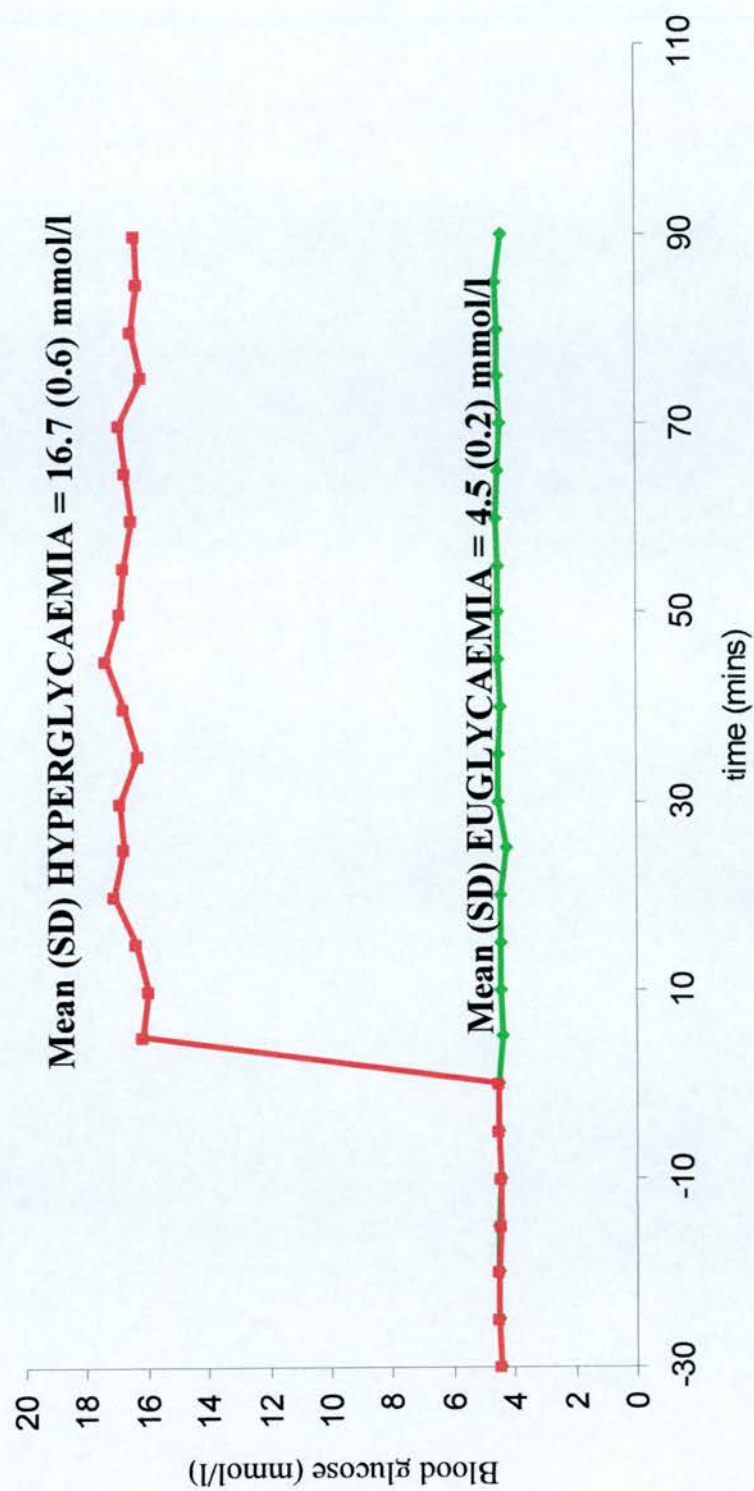
Figure 10.1: Study design

RESULTS

A stable blood glucose plateau was achieved during each study condition (Figure 10.2). The mean (SD) arterialised blood glucose concentration during the euglycaemia condition was 4.5 (0.2) mmol/l, and during the hyperglycaemia condition was 16.7 (0.6) mmol/l. Statistical analysis revealed that no significant order effects had occurred for any of the outcome variables of this study, and no significant gender differences were evident.

Figure 10.2: Mean blood glucose profiles during euglycaemia and hyperglycaemia studies.

n=20



Tests of Information Processing

The results of these tests are summarised in Table 10.1. During acute hyperglycaemia, performance in both the Trail Making B ($p=0.037$, $\eta^2=0.22$) and the Digit Symbol tests ($p=0.029$, $\eta^2=0.24$) was significantly impaired. The Four Choice Reaction Time score deteriorated during hyperglycaemia ($p<0.0001$, $\eta^2=0.56$). By contrast, the score for the Simple Reaction Time test was not significantly affected ($p=0.101$).

Tests of Memory

The results of the memory function tests are summarised in Table 10.2.

Immediate Memory

Acute hyperglycaemia had no significant effect on tests of immediate verbal (AVLT: $p=0.085$, Logical Memory: $p=0.162$) or immediate visual memory (Visual Reproduction: $p=0.515$, BVRT: $p=0.296$).

Delayed Memory

Tests of delayed verbal (AVLT Delayed: $p=0.26$, Logical Memory Delayed: $p=0.092$) and delayed visual memory (Visual Reproduction Delayed: $p=0.075$) were also not significantly impaired during hyperglycaemia.

Working Memory

Digit Span Backwards ($p=0.013$, $\eta^2=0.3$) and Letter/Number Sequencing tests ($p=0.023$, $\eta^2=0.25$) demonstrated a significant decrement during

hyperglycaemia. Performance in the Digit Span Forwards test was not significantly affected ($p=0.33$).

Tests of Attention

The results of the tests of attention are summarised in Table 10.3.

Visual Selective Attention

The mean number (SD) of map symbols circled during euglycaemia in one minute was 35.0 (6.7) versus 32.2 (5.1) during hyperglycaemia ($p=0.036$, $\eta^2=0.22$). The mean number (SD) of symbols circled in two minutes was also lower at 62.5 (8.7) during hyperglycaemia, compared with 64.8 (7.4) in the euglycaemia condition. However, the difference did not achieve statistical significance ($p=0.31$). In the telephone search task, no difference was demonstrated between euglycaemia and hyperglycemia in the number of symbols located (Table 10.3). The mean (SD) time taken to complete the task increased from 58.7 (8.3) seconds during euglycaemia, to 67.0 (13.1) seconds during hyperglycaemia ($p=0.043$, $\eta^2=0.27$) (Table 10.3).

Auditory Selective Attention

In the auditory elevator test with reversal, the achieved score declined from a mean (SD) of 5.3 (1.7) during euglycaemia to 4.4 (1.4) during hyperglycaemia ($p=0.014$, $\eta^2=0.29$). By contrast, the score attained on the elevator test with distraction did not deteriorate during hyperglycaemia, with a mean (SD) score of 8.8 (1.1) during euglycaemia compared to 8.3 (1.3) during hyperglycaemia ($p=0.117$).

Sustained Attention

Sustained attention did not deteriorate during hyperglycaemia using either the lottery ticket test or the elevator counting test (Table 10.3).

Attentional Switching

In the visual elevator task, no difference was observed in the raw score between the two study conditions. The mean (SD) raw score during euglycaemia was 8.9 (1.1) compared with 8.5 (0.9) during hyperglycaemia ($p=0.173$). However, a significantly longer time was required to complete each switch of the visual elevator task during hyperglycaemia with a mean (SD) time of 4.7 (0.9) seconds per switch, compared to 4.3 (1.0) seconds per switch during euglycaemia ($p=0.001$, $\eta^2=0.49$).

Divided attention

In the task that involved search of a telephone directory while counting, no significant difference was observed in the number of symbols that were located during either study condition (Table 10.3). The time taken to complete the task was higher during hyperglycaemia with a mean (SD) of 67.2 (17.1) seconds compared to 62.3 (9.5) seconds during euglycaemia, but this difference was not significant ($p=0.1$). The time per target score, which is the ratio of the number of circled symbols divided by the time taken for the task, was significantly higher during acute hyperglycaemia, with a mean (SD) of 1.2 (0.6) seconds/target during hyperglycaemia compared to 1.0 (0.6) seconds/target during euglycaemia ($p=0.049$, $\eta^2=0.2$).

Table 10.1: Results of tests of information processing during euglycaemia and hyperglycaemia in 20 adults with type 2 diabetes. Results are shown as mean (SD).

Subtest	Euglycaemia	Hyperglycaemia	<i>p</i> value	<i>Eta</i> ²
Digit Symbol Substitution test	70.2 (8.4)	66.8 (7.8)	.029	.24
Trail Making B test	40.9 (7.8)	43.9 (8.0)	.037	.23
Simple Reaction Time test	359.9 (84.6)	373.7 (72.3)	.101	.14
Four-Choice Reaction Time test	710.0 (116.7)	775.7 (122.9)	< .0001	.56

SD – Standard Deviation

Table 10.2: Results of tests of memory during euglycaemia and hyperglycaemia in 20 adults with type 2 diabetes. Results are shown as mean (SD).

Subtest	Euglycaemia	Hyperglycaemia	<i>p</i> value	<i>Eta</i> ²
Immediate Logical Memory	27.2 (4.3)	26.1 (4.1)	.162	.11
Immediate AVLT	35.0 (5.7)	32.4 (4.9)	.085	.16
Benton Visual Retention test	6.2 (1.2)	5.9 (0.9)	.296	.06
Visual Reproduction	76.9 (7.6)	77.9 (7.5)	.515	.02
Digit Span Forwards	9.6 (1.3)	9.2 (1.6)	.33	.05
Digit Span Backwards	8.6 (1.5)	7.9 (1.3)	.013	.30
Letter/Number Sequencing	10.4 (1.9)	9.4 (1.6)	.023	.25
Delayed Logical Memory	13.2 (2.1)	12.3(2.2)	.092	.15
Delayed AVLT	8.2 (2.2)	7.8 (1.9)	.26	.07
Delayed Visual Reproduction	14.1 (9.2)	10.8 (8.2)	.075	.17

AVLT – Auditory Verbal learning Test

SD – Standard Deviation

Table 10.3: Results of tests of attention during euglycaemia and hyperglycaemia in 20 adults with type 2 diabetes. Results are shown as mean (SD).

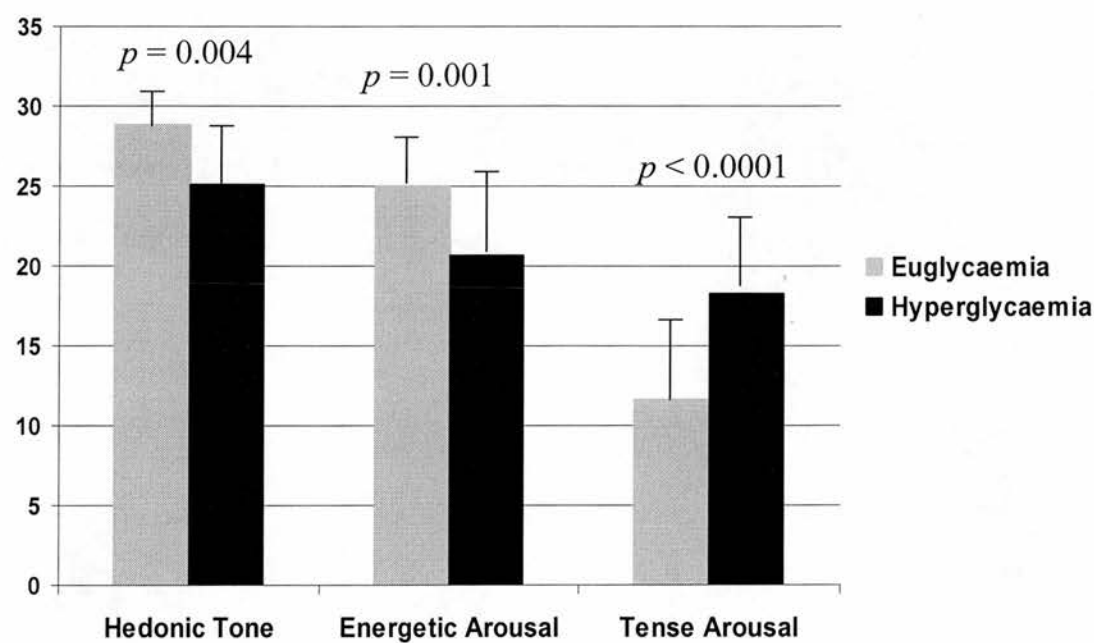
Subtest	Euglycaemia	Hyperglycaemia	<i>p</i> value	<i>Eta</i> ²
Map Search 1 minute	35.0 (6.7)	32.2 (5.1)	.036	.22
Map Search 2 minutes	64.8 (7.4)	62.5 (8.7)	.314	.06
Elevator Counting	6.6 (0.68)	6.7 (0.67)	.795	.01
Elevator Counting with distraction	8.8 (1.1)	8.3 (1.3)	.117	.13
Elevator Counting with reversal	5.3 (1.7)	4.4 (1.4)	.014	.29
Visual Elevator raw score	8.9 (1.1)	8.5 (0.9)	.173	.10
Visual Elevator switch time	4.3 (1.0)	4.7 (0.9)	.001	.49
Telephone Search raw score	3.6 (0.7)	3.7 (0.6)	.340	.05
Telephones Search total time	58.7 (8.3)	67.0 (13.1)	.043	.27
Telephone Search with counting	1.0 (0.6)	1.2 (0.6)	.049	.20
Telephone Search with counting total time	62.3 (9.5)	67.2 (17.1)	.100	.12
Telephone Search with counting time per target	1.0 (0.6)	1.2 (0.6)	.049	.20
Lottery	9.6 (0.7)	9.7 (0.6)	.630	.01

SD – Standard Deviation

Mood

The results of the mood questionnaire are shown in Figure 10.3. Hedonic Tone ($p=0.004$, $\eta^2=0.38$) and Energetic Arousal ($p=0.001$, $\eta^2=0.49$) scores were significantly lower during hyperglycaemia, while feelings of Tense Arousal were greater ($p<0.0001$, $\eta^2=0.56$).

Figure 10.3: Graph showing mean (SD) scores of the UWIST mood adjective checklist during euglycaemia and hyperglycaemia.



DISCUSSION

Relatively few studies have examined in detail the effects of acute hyperglycaemia on cognitive function, and non-cognitive functions such as mood, in people with diabetes (99,239-242,278). Earlier studies have provided inconsistent results. In view of the paucity of data on the effects of hyperglycaemia on these important aspects of everyday life, the present study has attempted a broad examination of the effects of hyperglycaemia on an extensive battery of cognitive tests, and mood state.

The present study has demonstrated that acute hyperglycaemia significantly impairs cognitive function and has a significant effect on mood in people with type 2 diabetes. We acknowledge that, with the administration of such an extensive battery of cognitive tests, there is the potential for type 1 statistical error, and ideally this should have been excluded using bonferroni statistical analysis.

The changes in mood that were observed during acute hyperglycaemia included increased feelings of agitation and anxiety (increased tense arousal), increased feelings of tiredness and lethargy (decreased energetic arousal) and decreased feelings of happiness (decreased hedonic tone). Changes in mood can influence cognitive performance. Further analysis of the cognitive test results with mood as a covariate demonstrated that the impairment in cognitive function occurred independently of changes in mood state.

In tests of information processing, performance in the Digit Symbol and Trail Making B tests was significantly impaired during acute hyperglycaemia. In the reaction time test there was no significant decrement in performance in the Simple

Reaction Time test. However, performance in the more complex Four-Choice Reaction Time test deteriorated very significantly during hyperglycaemia.

There was no significant disruption of test performance in the relatively simple tests of short-term verbal (Logical Memory, AVLT) and short-term visual (Visual Reproduction, BVRT) memory during hyperglycaemia. Tests of delayed memory were also unaffected.

The Digit Span test is a test of working memory. Digit Span Forwards test performance was not significantly impaired during acute hyperglycaemia. However, performance in the more complicated Digit Span Backwards test showed a significant decrement during hyperglycaemia. Performance in the Letter/Number Sequencing test, another complicated test of working memory, was also significantly worse during acute hyperglycaemia.

Scores in the Lottery and Elevator Counting tests, tests of sustained attention, were not significantly affected by hyperglycaemia. However, there was a significant reduction in performance in the more difficult task of Elevator Counting with Reversal during acute hyperglycaemia.

In the Map Search, a test of visual selective attention, significantly fewer symbols were marked after one minute during hyperglycaemia compared to euglycaemia. There was no significant difference in the overall two minute score. This was due to a 'ceiling effect' after two minutes. There was no difference in the total number of symbols located in the Telephone Search test, but the time taken to complete the task was significantly longer during hyperglycaemia compared to euglycaemia. Similarly, in the Telephone Search While Counting test, although there was no significant difference in the total number of symbols located during

either study condition, the time taken to complete the test was significantly longer during hyperglycaemia. In the Visual Elevator task, no significant difference was observed in the raw score during hyperglycaemia compared to euglycaemia. However, the time taken to complete each switch was significantly longer during hyperglycaemia.

The adverse effects of acute *hypoglycaemia* on cognitive function are well established. The results of the present study indicate that acute *hyperglycaemia* also has a significant deleterious effect on neuropsychological function. With the exception of the Four-Choice Reaction Time test, the η^2 values for the tests of cognitive function are modest. Nevertheless, the effects of hyperglycaemia on mood and some tests of cognitive function were significant. From the cognitive function test results it was apparent that acute hyperglycaemia caused impairment in performance of complex tasks of cognitive function in people with type 2 diabetes. This finding is in agreement with the results from an earlier study by Davis *et al* (240), who demonstrated that the impairment of cognitive function in children with type 1 diabetes during acute hyperglycaemia was limited to complex tests of cognitive ability. We have also demonstrated impairment of performance during acute hyperglycaemia in tests that require a speeded response, suggesting that accuracy is preserved at the expense of speed. It is not known whether the effect of hyperglycaemia on cognitive function or mood is sustained or protracted, and this will require further investigation. The effects of acute hypoglycaemia on cognitive function have been observed to return to normal within two hours after euglycaemia is restored (95,100).

The precise mechanism by which acute hyperglycaemia may cause cognitive dysfunction is unclear. It is possible that acute hyperglycaemia may cause structural or functional changes in the blood brain barrier, may influence synthesis or re-uptake of neurotransmitters, including neurotoxic neurotransmitters such as glutamate, or acute hyperglycaemia may induce cytotoxic osmotic changes within neurones.

There is already some indirect evidence to suggest that hyperglycaemia has adverse effects on cerebral function. There is increasing evidence to suggest that chronic hyperglycaemia, as indicated by surrogate markers such as polyneuropathy or retinopathy, is involved in the pathogenesis of the cognitive impairment associated with diabetes (186,307,308), and acute hyperglycaemia has been shown to enhance cerebral damage resulting from ischemic stroke (308).

Our findings are in concordance with anecdotal reports from people with diabetes, and are supported by a recent study conducted by Cox *et al* (309) who have demonstrated changes in mood and impairment in cognitive function during acute hyperglycaemia in people with both type 1 or type 2 diabetes. The findings of our study are of considerable practical importance to people with type 2 diabetes in whom moderate hyperglycaemia is common. Such effects of hyperglycaemia on cognitive function may significantly interfere with many activities of daily living and may influence therapeutic strategies aimed at treating postprandial hyperglycaemia.

PART V

HYPOGLYCAEMIA AND VASCULAR HAEMODYNAMIC FUNCTIONS

Chapter 11

Vessel Wall Stiffness in Type 1 Diabetes, and the Central Haemodynamic Effects of Acute Hypoglycaemia

INTRODUCTION

Central arterial pressure and waveform convey important information about cardiovascular status (244,249). Arterial blood pressure is usually recorded non-invasively from the brachial artery by sphygmomanometry. It is assumed that the pressure is the same throughout the arterial tree and that it represents an accurate index of aortic peak pressure and of left ventricular systolic pressure, and so of left ventricular afterload. However, pressure values in the peripheral circulation are an inaccurate measure of central pressure because of amplification of the pressure pulse between central and peripheral arteries (245,265).

Each left ventricular contraction generates a pressure wave which travels along the major arteries until it meets peripheral resistance, at which point the wave is reflected back to the heart. Arterial stiffness influences the velocity of the reflected pressure wave. Normally, the reflected wave reaches the heart during diastole and, by increasing diastolic pressure, coronary perfusion is enhanced. However, where arterial stiffening develops, the increased pulse wave velocity of the stiffened arteries leads to an earlier reflection of the wave so that it reaches the heart during late systole (249,261). The increase in systolic pressure that results from earlier reflection of the pressure wave is referred to as “augmentation”. The Augmentation Index (AIx) is the augmentation pressure expressed as a percentage of the pulse pressure. AIx increases with increasing age, and also increases in disease states such as hypertension (262). It is also influenced by heart rate; a decrease in AIx is observed with increasing heart rate (264).

Arterial stiffness, is an independent predictor of cardiovascular morbidity, especially for myocardial infarction (249-253). The development of pulse wave analysis has allowed the measurement of central arterial pressure, and the degree of its augmentation by pulse wave reflection, to be studied reliably and non-invasively (254-256). The system utilises applanation tonometry, with the external application of a micromanometer-tipped probe to record peripheral pulse waveforms. The radial artery is usually used for these measurements because of its close proximity to, and support provided by, nearby bony structures (258,259).

Diabetes is a chronic disorder, associated with the development of microvascular and macrovascular disease as a result of long-term exposure to hyperglycaemia (310,311). Hence, diabetes is associated with a high prevalence of macrovascular disease causing life-threatening vascular events, such as acute myocardial infarction and stroke.

Several studies have examined arterial stiffness in people with diabetes. Arterial stiffness has consistently been found to be increased in people with Type 2 diabetes (312,313), and also in their relatives (310), but the results of studies that have examined arterial stiffness in people with Type 1 diabetes have been inconsistent. Some studies have demonstrated increased levels of arterial stiffness in people with Type 1 diabetes compared to non-diabetic controls (315-317), whereas other studies have shown either no difference (318) or *reduced* arterial stiffness (319) in people with Type 1 diabetes. The discrepancy between results may be due to methodological differences between studies. Participants have varied in their duration of diabetes, their age and gender, smoking status, peripheral blood pressure

and serum cholesterol concentration and have differed in the presence or absence of microvascular complications.

Hypoglycaemia is a common side-effect of insulin therapy in people with diabetes (1,173). To protect the function and integrity of the central nervous system, a hierarchy of responses are activated when blood glucose falls below 4.0 mmol/l which help to restore normoglycaemia (6,320). A fall in blood glucose activates the autonomic system via hypothalamic autonomic centres in the brain, so stimulating the sympatho-adrenal system and the secretion of catecholamines, particularly adrenaline (epinephrine). This profound autonomic stimulus provokes haemodynamic changes, including an increase in heart rate and stroke volume, increased myocardial contractility and a rise in cardiac output (50). Left ventricular ejection fraction has been shown to increase by almost a third in response to acute hypoglycaemia (48). In addition, peripheral blood pressure is affected with an increase in systolic and decrease in diastolic pressure, while mean arterial pressure remains unchanged.

There is some evidence that insulin *per se* has a direct effect on large artery function. In non-diabetic humans, intravenous infusion of insulin has been observed to be associated with a reduction in AIx, suggesting that insulin increases the distensibility (i.e. reduces the stiffness) of large arteries (321). In subjects with insulin resistance, a characteristic of Type 2 diabetes, the ability of insulin to decrease arterial stiffness is impaired (322).

The present study aimed to examine arterial wall stiffness in people with and without Type 1 diabetes of varying duration, to determine the central haemodynamic

responses to intravenous insulin and acute hypoglycaemia in these subjects, and to ascertain whether these responses are modified by the presence of Type 1 diabetes.

METHODS

Subjects

To avoid potential gender differences in the counterregulatory hormonal responses to hypoglycemia (320), three groups of male subjects, matched for age, body mass index (BMI) and peripheral blood pressure, were studied. 10 non-diabetic volunteers (Group 1), 10 subjects with Type 1 diabetes of short duration (<5 years) (Group 2) and 10 subjects with Type 1 diabetes of longer duration (>15 years) (Group 3). Subjects with diabetes were matched for HbA1c, and were recruited from the out-patient clinic at the Department of Diabetes at the Royal Infirmary of Edinburgh. Blood pressure and plasma cholesterol concentration were measured before inclusion in the study. HbA1c was measured by high performance liquid chromatography (Variant II Hemoglobin Testing System, Biorad Diagnostics Group, Hercules, California, USA), with a local DCCT-adjusted non-diabetic reference range of 4.3-6.5%. Subjects were excluded from the study if there was a history of hypertension (BP>140/80mmHg), hypercholesterolemia (total cholesterol >5.0mmol/l) or other chronic disease, or a history of previous head injury, alcohol or drug abuse, seizure or blackouts. All the subjects were non-smokers, had no intercurrent illness and were not taking any regular medication (other than insulin for diabetes). Subjects with diabetes were excluded if they had any evidence of microvascular disease, other than background diabetic retinopathy (two subjects in Group 2 and five subjects in Group 3). The presence of retinopathy was determined by direct ophthalmoscopy, peripheral neuropathy by clinical examination, and nephropathy by the presence of microalbuminuria (albumin:creatinine ratio >2.5

mg/mmol for women and >3.5 mg/mmol for men). All potential subjects underwent standard autonomic function testing (323) prior to inclusion in the study and any with autonomic dysfunction were excluded. Subjects were also excluded if they had a history of impaired awareness of hypoglycemia.

Studies were postponed if a subject had experienced hypoglycemia in the preceding 48 hours, and subjects were required to measure their blood glucose frequently during this period, including bedtime tests on the evening before the study to identify biochemical hypoglycemia. Ethical permission for the study was given by the Lothian Research Ethics Committee. All subjects gave their written informed consent.

Procedure

Each study session began at 08.00 after a 10-12 hour overnight fast. The subjects with type 1 diabetes omitted their insulin on the morning of the study. The study design is shown in Figure 11.1. A hyperinsulinaemic glucose clamp (238) was used, initially, to maintain euglycaemia (blood glucose concentration 4.0-7.0 mmol/l) for 20 mins before the induction of acute hypoglycemia. As the purpose of inducing acute hypoglycemia was to provoke an acute autonomic reaction with profound activation of the sympatho-adrenal system, a rapid fall in blood glucose was then induced by infusing insulin intravenously at a rate of 2mU/kg/min. This caused a controlled decline in blood glucose to a level which triggered the autonomic reaction associated with hypoglycemia. This method of inducing hypoglycemia stimulates the development of hypoglycemia under everyday conditions and can not be induced using the hyperinsulinaemic glucose clamp technique. Any variability between

subjects in the rate of fall in blood glucose and the depth of the hypoglycemia achieved is inconsequential as the intention is to lower blood glucose to the blood glucose threshold at which autonomic activation is stimulated.

The autonomic reaction (designated as R) was identified by an abrupt rise in heart rate (15% above baseline), the rapid onset of autonomic symptoms, such as sweating and tremor, and a rapid rise in peripheral systolic blood pressure. When the autonomic reaction occurred the clock timer was reset to zero and all subsequent measurements were timed relative to this point. This accommodates the variable time interval that occurs between non-diabetic and diabetic individuals in development of the autonomic reaction using the insulin infusion technique (324). As soon as R had occurred, the insulin infusion was discontinued and hypoglycemia was reversed using an intravenous infusion of 20% dextrose to restore normoglycaemia. All subjects consumed a meal on completion of the study.

Measurements:

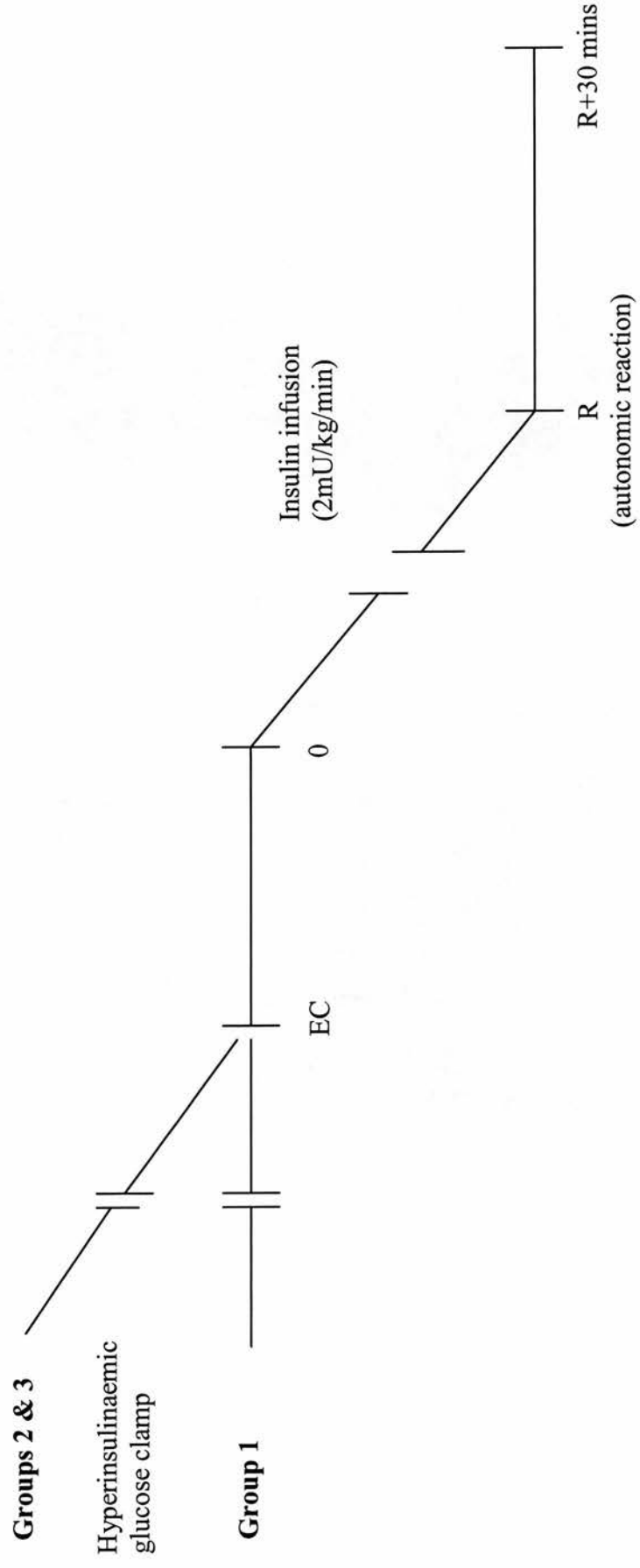
During the study, arterialised blood glucose was measured at the bedside (Yellow Springs Instrument 2300 Stat, Yellow Springs, Ohio, USA) every 5 minutes until R, and every 15 minutes thereafter until R+30 minutes. The maximal haemodynamic changes in response to hypoglycemia occur in the 30 minutes following the onset of the autonomic reaction (R) (48). Pulse wave analysis at the radial artery was therefore performed every 5 minutes until R, and every 5 minutes thereafter until R+30 minutes. Heart rate was monitored continuously using a pulse oximetry probe. Peripheral blood pressure was recorded every 5 minutes using a digital automated sphygmomanometer.

The Edinburgh Hypoglycaemia Scale (276), a validated subjective self-rating questionnaire, was used to document the symptoms of hypoglycemia experienced by the subjects during the study. The symptoms of hypoglycemia were classified as autonomic (hunger, palpitations, sweating, shaking), neuroglycopenic (drowsiness, confusion, inability to concentrate, speech difficulty, blurred vision) and non-specific (malaise, nausea, headache). Each symptom was graded on a Likert Scale of 1 to 7 (1 = not present, 7 = very intense). The questionnaire was applied at baseline and at 5 minute intervals thereafter until the onset of the autonomic reaction. It was repeated at R+30 minutes.

Statistics

A general linear model (repeated measures analysis of variance) was used to compare means within individual groups. Between groups, an ANOVA with post-hoc t-tests was used. Correlation analyses for changes in AIx and insulin dose were performed using Pearson's correlation. A *p* value < 0.05 was considered to be significant. All analyses were performed using SPSS version 11.0 for Windows. All results are expressed as mean \pm SD.

Figure 11.1: Study design.



RESULTS

The clinical characteristics of the subjects in the study are shown in Table 11.1.

Changes in blood glucose concentration

The blood glucose concentrations (mean \pm SD) at baseline were 4.3 \pm 0.5 mmol/l in Group 1 (non-diabetic) and 8.4 \pm 2.1 mmol/l and 8.9 \pm 3.1 mmol/l in Groups 2 and 3 (diabetic), respectively. Following application of the euglycaemic clamp, the blood glucose concentrations were stabilised at 4.2 \pm 0.1 mmol/l (Group 1), 4.3 \pm 0.2 mmol/l (Group 2) and 4.3 \pm 0.2 mmol/l (Group 3). The nadir blood glucose concentrations at R were 2.9 \pm 0.3 mmol/l (Group 1), 2.8 \pm 0.3 mmol/l (Group 2) and 2.6 \pm 0.3 mmol/l (Group 3).

Peripheral blood pressure

No significant differences were observed in the peripheral systolic, diastolic, and mean arterial pressures between the three groups at baseline. In all three groups of subjects an increase in peripheral systolic blood pressure and a decrease in peripheral diastolic pressure occurred at R, compared to baseline (Table 11.2). Systolic and diastolic pressures had returned to baseline levels by R+30 minutes in all three groups (Table 11.2).

Central arterial pressure

Central systolic blood pressure at baseline was significantly higher in Group 3 compared to Groups 1 and 2 (Table 11.1). A small, but significant, reduction in central systolic pressure was observed at R in all three groups, compared to the respective baseline levels (Table 11.2). No change in central diastolic blood pressure was observed throughout the study in any of the three groups (Table 11.2).

Heart rate and autonomic symptom score

The induction of hypoglycaemia and the onset of the autonomic reaction (R) was associated with significant increments of heart rate and autonomic symptom score in all three groups compared to baseline levels (Table 11.2). In all groups, the heart rate and the autonomic symptom score had returned to baseline values at R+30 minutes.

Augmentation index (AIx)

At baseline, no difference in AIx was discernible between Group 1 and Group 2, whereas the AIx in Group 3 was higher than in either of these groups (Table 11.1).

All three groups exhibited a progressive decline in AIx between baseline and the development of hypoglycaemia at R (Figure 11.2). At R+30 minutes, AIx had risen to approach baseline levels in all three groups.

Changes in AIx and insulin dose

There was no significant difference between the amount of insulin infused during the studies in the three different groups (Table 11.1). However, amongst the

separate groups, there was a significant correlation between changes in AIx and insulin exposure. A greater decrement in AIx was associated with greater insulin exposure (Group 1 $r = 0.30$ $p = 0.05$, Group 2 $r = 0.34$ $p = 0.04$, Group 3 $r = 0.3$ $p = 0.03$).

Table 11.1: Patient characteristics. Group 1: Non-diabetic subjects. Group 2: Type 1 diabetes <5 years. Group 3: Type 1 diabetes >15 years. Results are expressed as mean (SD).

	Group 1 n=10	Group 2 n=10	Group 3 n = 10	ANOVA
Age (years)	29.4 (4.4)	30.2 (7.9)	31.1 (4.7)	p=0.8
Duration of diabetes (years)		3.0 (1.1)	18.4 (0.7)	p=0.3
HbA1c		7.6 (0.7)	8.1 (0.3)	p=0.2
Body Mass Index (kg/m ²)	24.0 (1.2)	24.2 (1.1)	24.4 (1.4)	p=0.9
Total plasma cholesterol (mmol/l)	4.5 (0.8)	4.7 (0.6)	4.8 (0.6)	p=0.4
Peripheral systolic BP (mmHg)	121.7 (10.5)	123.3 (6.5)	125.9 (9.8)	p=0.3
Peripheral diastolic BP (mmHg)	65.6 (7.4)	69.4 (7.7)	72.7 (6.7)	p=0.2
Mean arterial pressure (mmHg)	92.4 (8.7)	87.5 (9.1)	92.5 (9.1)	p=0.4
Central systolic BP (mmHg)	114.4 (14.9)	110.9 (8.0)	121.5 (9.7)	p<0.05
Central Diastolic BP (mmHg)	77.2 (10.5)	74.8 (8.4)	79.0 (8.7)	p=0.6
Augmentation index (AIx)	9.3 (12.4)	10.0 (8.3)	21.8 (6.9)	p<0.05
Total insulin dose (Units)	14.6 (4.4)	12.7 (3.1)	15.7 (4.8)	p = 0.6

Post hoc t tests

Central systolic pressure: Group 3 vs. Group 1 p = 0.04, Group 3 vs. Group 2 p = 0.047

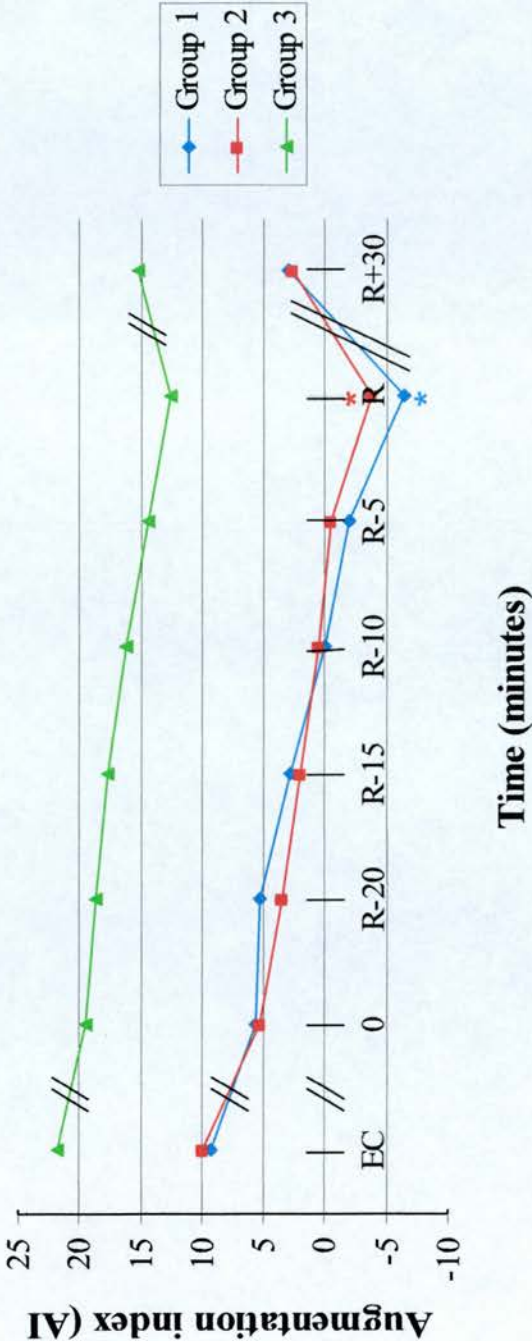
Augmentation index: Group 3 vs. Group 1 p = 0.02, Group 3 vs. Group 2 p = 0.002

BP = blood pressure HbA1c = glycated haemoglobin (non-diabetic range 4.3-6.5%)

Table 11.2: Haemodynamic measurements. Group 1: Non-diabetic subjects. Group 2: Type 1 diabetes <5 years. Group 3: Type 1 diabetes >15 years. Results are expressed as mean (SD). Results marked * represent a significant within group difference ($p<0.05$). EC = euglycaemic clamp commenced. 0 = start of insulin infusion to induce hypoglycemia. R = autonomic reaction.

		Group 1 n = 10	Group 2 n = 10	Group 3 n = 10
Peripheral systolic blood pressure (mmHg)	EC	121.7 (10.5)	123.3 (6.5)	125.9 (9.8)
	0	121.1 (9.7)	117.8 (9.4)	131.8 (11.2)
	R	*143.5 (11.8)	*137.8 (9.8)	*144.5 (7.9)
	R+30	116.9 (11.0)	114.6 (12.2)	130.6 (8.1)
Peripheral diastolic blood pressure (mmHg)	EC	65.6 (7.4)	69.4 (7.7)	72.7 (6.7)
	0	68.1 (6.1)	68.7 (7.0)	72.5 (7.1)
	R	*61.6 (6.5)	*63.6 (9.9)	*68.7 (3.6)
	R+30	66.5 (11.2)	67.4 (14.6)	71.1 (6.7)
Mean arterial pressure (mmHg)	EC	92.4 (8.7)	87.5 (9.1)	92.5 (9.1)
	0	92.6 (6.5)	88.1 (8.1)	92.9 (8.8)
	R	92.3 (7.8)	87.7 (7.9)	93.9 (11.2)
	R+30	92.3 (8.5)	87.43 (9.0)	89.6 (12.0)
Central systolic blood pressure (mmHg)	EC	114.4 (14.9)	110.9 ((8.0)	120.5 (9.2)
	0	114.2 (12.6)	109.9 (7.2)	123.8 (6.4)
	R	*110.8 (13.9)	*108.1 (7.0)	*118.0 (7.7)
	R+30	113.5 (5.6)	110.5 (9.2)	122.8 (7.8)
Central diastolic blood pressure (mmHg)	EC	77.2 (10.5)	74.8 (8.4)	79.0 (8.7)
	0	77.3 (11.1)	75.1 (7.4)	79.1 (8.1)
	R	77.3 (10.4)	74.8 (8.8)	78.7 (8.9)
	R+30	76.9 (8.9)	74.4 (9.0)	78.9 (8.2)
Heart rate (beats/min)	EC	60.5 (11.7)	66.5 (7.1)	70.7 (7.8)
	0	60.2 (11.1)	66.3 (9.0)	68.4 (10.1)
	R	*76.7 (12.8)	*82.2 (8.4)	*79.0 (7.1)
	R+30	59.1 (10.2)	66.2 (9.7)	66.8 (8.3)
Autonomic symptom score	EC	0.2 (0.2)	0.5 (0.4)	0.2 (0.1)
	0	0.4 (0.6)	0.6 (0.3)	0.8 (0.6)
	R	*11.2 (4.4)	*9.9 (5.1)	*7.9 (1.4)
	R+30	0.5 (0.3)	0.8 (0.8)	0.9 (1.1)

Figure 11.2: Mean change in augmentation index. Group 1: Non-diabetic subjects. Group 2: Type 1 diabetes <5 years. Group3 : Type 1 diabetes >15 years. EC = euglycaemic clamp commenced. 0 = start of insulin infusion to induce hypoglycaemia. R = autonomic reaction. # = $p<0.01$ compared to values at EC. * = $p<0.0001$ compared to values at 0.



DISCUSSION

Arterial stiffness is increased in people with Type 2 diabetes (312,313,325,326), in their non-diabetic relatives (314) and in people with impaired glucose tolerance (327). In people with Type 1 diabetes results have been discrepant. Some studies have shown increased arterial stiffness compared to non-diabetic controls (315-317), whereas others have shown either no difference (318), or reduced arterial stiffness (319). These differences may relate to the methodologies used and to patient selection, with participants differing in their duration of diabetes, age and gender, smoking status, peripheral blood pressure, serum cholesterol concentrations and the presence or absence of microvascular disease.

Here, arterial stiffness and central arterial pressure were studied in both non-diabetic adults and in people with type 1 diabetes of differing duration, using radial artery pulse wave analysis and calculation of augmentation index, and the subjects, all of whom were non-smokers, were matched for age, gender, blood pressure, serum cholesterol and body mass index. The results showed no difference in vessel wall stiffness or central arterial pressure between a group with well-controlled type 1 diabetes of short duration and a matched cohort of healthy non-diabetic volunteers. However, the people with type 1 diabetes of longer duration had greater stiffness of the arterial vessel wall and an elevated central arterial pressure compared to non-diabetic controls. This suggests that the duration of exposure to chronic hyperglycemia influences vessel wall stiffness, rather than the presence of type 1 diabetes itself. This may possibly result from structural changes caused by the gradual deposition of advanced glycation endproducts (AGEs) within the walls of

large blood vessels (328). The endothelium (329,330) and arterial wall smooth muscle bulk and tone (331) (the latter under the control of the endothelium) also influence the elasticity of arteries, and it is also possible, therefore, that increased vessel wall stiffness in people with type 1 diabetes of long duration is the result of functional changes that occur within the endothelium with increasing duration of diabetes.

In the present study, the rise in peripheral systolic blood pressure that is known to accompany acute insulin-induced hypoglycaemia was accompanied by a *reduction* of central systolic blood pressure. These findings highlight the potential for divergent responses of central and peripheral arterial pressures, and support the premise that the measurement of peripheral blood pressure by conventional methods does not provide an accurate measure of the central arterial pressure load on the left ventricle. Specifically, the results show how the changes of central and peripheral arterial pressures differ in response to the stress provoked by acute hypoglycaemia and mediated by sympatho-adrenal stimulation. In the present study the difference that was observed between the responses of peripheral and central systolic blood pressures to insulin-induced hypoglycemia may be a consequence of the observed changes in augmentation index; a reduction in AIx produces diminished amplification of the systolic pressure wave and so causes a net reduction overall in central arterial systolic blood pressure. It is possible that this observed change in augmentation index had resulted either from changes in heart rate (264) or from the actions of vasoactive hormones that are secreted in response to hypoglycemia (332), or as a combination of both effects. An increment in heart rate of 10 beats per minute, causes a fall in augmentation index of around 4% (264), and the infusion of

noradrenaline promotes an increase in augmentation index (333). However, in the present study, the augmentation index was observed to fall initially during euglycaemia, before any stimulated changes in heart rate, autonomic symptom score or peripheral blood pressure. Insulin *per se* may have a direct effect on the functional integrity of large blood vessels. In support of this, it was observed that amongst individuals within each study group, there was a significant correlation between changes in AIx and the amount of insulin infused. The early changes in AIx may therefore be a consequence of the direct action of insulin on endothelial function or smooth muscle tone of large arteries. This interpretation would be consistent with the results of other studies that have demonstrated that the intravenous administration of insulin increases the distensibility of large arteries (321,322).

The decrement of augmentation index was less in the group who had Type 1 diabetes of longer duration compared with those with diabetes of short duration and to non-diabetic subjects. This would be consistent with greater stiffness and reduced elasticity of the vessel walls of large arteries in people with Type 1 diabetes of long duration. Consistent with this, Westerbacka *et al* (334) have shown that the effect of insulin to reduce vessel wall stiffness in large arteries is diminished in people with Type 1 diabetes who have no vascular complications. The mean duration of Type 1 diabetes in their participants was 18 years (334), which is similar to the duration of diabetes in Group 3 of the present study. We acknowledge that the algorithm for relating peripheral and central pressures by applanation tonometry was derived in non-diabetic subjects. However, in this study, the changes in the central haemodynamic responses to insulin infusion and the induction of acute hypoglycemia were similar amongst both the non-diabetic and the diabetic groups.

Type 1 diabetes is associated with an increased prevalence of cardiovascular disease, and vessel wall stiffness is an independent predictor of cardiovascular morbidity and mortality. The present study has demonstrated that a greater duration of type 1 diabetes was associated with an increase in vessel wall stiffness, before the development of overt macrovascular disease. Central systolic pressure at rest was also greater in people with type 1 diabetes of longer duration, although no apparent difference was observed in peripheral blood pressure between the three study groups. Changes in brachial pressure may, therefore, underestimate changes in aortic pulse pressure and the response of left ventricular systolic function to haemodynamic stress in people who have had Type 1 diabetes for several years. These vascular changes and the attendant rise in central arterial blood pressure may contribute to the increased risk of cardiovascular morbidity in people with type 1 diabetes.

PART VI

CONCLUSIONS

Chapter 12

**Concluding Comments and Suggestions for Future
Research**

Hypoglycaemia is a very common occurrence in people with insulin-treated diabetes and, because the human brain is vitally dependent on glucose as its main source of energy, cerebral deprivation of glucose rapidly causes cognitive dysfunction through the direct effects of acute neuroglycopenia.

Hypoglycaemia, therefore, affords the unique opportunity to study cognitive processes under controlled deprivation of the brain's energy supply. Russell & Rix-Trot, in 1975 (237), observed that blood glucose concentrations below 3.0 mmol/l, occurring as a consequence of an intravenous bolus injection of insulin, were associated with impairment of cerebral function, and since then a large literature on the cognitive effects of acute hypoglycaemia has developed. Tasks that are complex, attention demanding and require a rapid response are more impaired during neuroglycopenia. In general, tests that involve attention, concentration, psychomotor skill, the accessing of long term memory and the ability to ignore distracting information, tend to deteriorate when blood glucose declines below about 3.0 mmol/L. Memory, one of the most important cognitive domains with respect to everyday function, has not, until now, been examined in detail during acute hypoglycaemia. In view of the relative lack of data on the effects of hypoglycaemia on this important cognitive domain, the present studies have attempted a comprehensive examination of the effects of acute hypoglycaemia on short-term, delayed and working memory using verbal and non-verbal materials.

These studies have conclusively demonstrated that acute hypoglycaemia significantly impairs a broad spectrum of memory functions both in people with, and in people without Type 1 diabetes. The domains of working memory and of delayed memory were particularly susceptible to the adverse effects of acute hypoglycaemia.

The results of these studies form a foundation for research which examines the biological and practical aspects of memory function in more detail. In the brain, the medial temporal lobe, the hippocampus and the parahippocampal apparatus are the principal structures involved with memory performance. It is known that the brain is susceptible to neuroglycopenia in a rostrocaudal direction; the cerebral cortex and hippocampus appear to be the areas most sensitive to the deleterious effects of acute hypoglycaemia. It could be hypothesised, therefore, that the hippocampus and memory processes would be particularly vulnerable to the harmful effects of hypoglycaemia. The results of the present study would support this postulation. Working memory tasks have been shown to exert high demands on brain extracellular glucose. The vulnerability of working memory to the effects of hypoglycaemia, therefore, may be a consequence of an interaction between local energy demands of the brain and the regional distribution of the glucose transport receptors GLUT1 and GLUT3.

The results of these studies also lays a foundation for more psychological research which should inquire, for example, whether the process of consolidating (laying down) new memories or retrieving already-established memories are particularly sensitive to the effects of hypoglycaemia.

Rehearsal of information allows material to be transferred from short-term memory to long-term memory. This process is referred to as *consolidation*. Through rehearsal, the neural activity responding to sensory stimulation can be sustained. Sustained activity causes permanent structural changes in the brain which are responsible for the formation of long-term memory. The profound impact of hypoglycaemia on delayed memory that has been demonstrated is likely to be a

consequence of the disruption, by neuroglycopenia, of the normal pathways of hippocampal neural activity that are responsible for the transfer of information from short-term to longer-term memory. It is possible, therefore, that short-term exposure to relatively moderate hypoglycaemia may disrupt the recent memory of events occurring immediately *before* exposure to hypoglycaemia.

Functional magnetic resonance imaging (fMRI) is a very sensitive form of neuroimaging that has the ability to detect subtle signs of neuronal activation and dysfunction. The technique of fMRI utilises Blood Oxygen Level Dependent (BOLD) contrast to measure blood flow and, indirectly, neural activity. It is able to detect changes in blood flow and oxygen consumption and this correlates closely with definable alterations in neuronal activity (335). During cognitive stimulation, blood flow and metabolism increase in specific areas of the brain and these changes can be detected using fMRI (336-340). Recently, the development of sophisticated and sensitive functional neuroimaging techniques in humans has confirmed the importance of medial temporal lobe structures, such as the hippocampus and adjacent parahippocampal regions, to memory function in normal individuals (284,285,341,342).

The neuroanatomical correlates of human memory can be examined using fMRI which can be applied to examine the brain regions involved in selective memory processes. A limited number of studies have utilised fMRI to study the effects of hypoglycaemia and one study has used fMRI to study patterns of brain activation in response to cognitive testing during hypoglycaemia (343). fMRI could therefore, be used to examine functional impairment of the medial temporal lobe, and

correlate brain activation aspects of memory, following exposure to acute hypoglycaemia.

Many studies have demonstrated the deleterious effects of acute *hypoglycaemia* on cognitive function and on mood states, but considerably less information is available on the effects of acute *hyperglycaemia* on cognitive function. Hyperglycaemia, however, commonly occurs as a consequence of the relative or absolute insulin deficiency that is intrinsic to diabetes and, therefore, any potential detrimental effects of hyperglycaemia on cognition would be of huge importance. In study 3, we have demonstrated that acute hyperglycaemia significantly impairs cognitive function and also has a significant effect on mood in people with type 2 diabetes. Performance in tasks of cognitive function that are particularly complex was most significantly impaired during acute hyperglycaemia. The changes in mood that were observed during acute hyperglycaemia included increased feelings of agitation and anxiety, increased feelings of lethargy and decreased feelings of happiness. Such effects of hyperglycaemia on cognitive function may significantly interfere with many activities of daily living for many people with diabetes. The findings from our study would suggest that the detrimental effects of acute hyperglycaemia are modest and it would be useful, therefore, to correlate this degree of cognitive impairment with performance in everyday tasks encountered in daily life.

Other potential areas of future research could include the ascertainment of the duration of cognitive impairment caused by acute hyperglycaemia, the precise mechanism by which acute hyperglycaemia causes cognitive dysfunction,

determination of whether there is a particular threshold of blood glucose above which the adverse effects of acute hyperglycaemia on cognitive function are manifest, and the potential impact of recent preceding exposure to acute hyperglycaemia. There may be potential for cerebral adaptation to occur in response to high prevailing blood glucose concentrations. The effect of recent antecedent hyperglycaemia, or of more prolonged exposure to hyperglycaemia in subjects with longstanding poor glycaemic control, may influence cognitive performance during acute hyperglycaemia.

In studies 4, 5 and 6 we have addressed the potential effect of Type 1 diabetes on arterial wall stiffness, which is an independent predictor of cardiovascular morbidity and mortality. Arterial stiffness has been studied previously in people with type 1 diabetes but the results have been inconsistent and variable. The present studies have demonstrated that people with well-controlled type 1 diabetes of short duration, who had no evidence of significant microvascular disease, had no demonstrable difference in vessel wall stiffness compared to a matched cohort of healthy volunteers. However, people with type 1 diabetes of longer duration did have greater stiffness of vessel walls compared to non-diabetic controls. This finding suggests that increasing duration of exposure to chronic hyperglycaemia results in increased vessel wall stiffness. The mechanism by which this is manifest is unclear, but it is possible that increased vessel wall stiffness is a consequence of the deposition of advanced glycation endproducts (AGEs) within the vessel wall. This may cause endothelial dysfunction or may promote the premature development of occult atherosclerosis.

Arterial blood pressure is usually recorded non-invasively from the brachial artery by sphygmomanometry. It is assumed that the pressure is the same throughout the arterial tree, that peripheral blood pressure is an accurate reflection of left ventricular afterload, and that it can be used therefore as an index of cardiovascular risk. The present study demonstrated that central systolic pressure was greater in people with type 1 diabetes of long duration, despite no apparent difference in the changes in peripheral blood pressure between the three study groups. Changes in brachial pressure may, therefore, underestimate changes in aortic pulse pressure and of left ventricular systolic stress in people with type 1 diabetes of long duration, and may therefore underestimate cardiovascular risk. These vascular changes may contribute to the increased cardiovascular risk of people with type 1 diabetes and determination of central arterial pressure and vessel wall stiffness may, therefore, provide a more accurate index of cardiovascular risk in patients with diabetes.

The results of the present study suggest that, in type 1 diabetes, it is the length of exposure to hyperglycaemia that influences vessel wall stiffness, rather than type 1 diabetes *per se*. It would be of interest to examine central arterial pressure and arterial wall stiffness in individuals with Type 1 diabetes who had developed microvascular complications. Assuming the presence of microvascular disease to be a surrogate of exposure to more profound hyperglycaemia, it could be hypothesised that such individuals would exhibit greater levels of arterial stiffness and central arterial pressure when compared to individuals with Type 1 diabetes with no microvascular complications, when subjects are matched for duration of diabetes. It is probable that the deleterious effects of hyperglycaemia on vessel wall stiffness are

cumulative and relate to both the duration of exposure to hyperglycaemia and the magnitude of hyperglycaemia.

Blood glucose is tightly regulated in health, and spontaneous hypoglycaemia in the non-diabetic individual is very rare. The majority of episodes of hypoglycaemia occur in people with insulin-treated diabetes and, for most people with insulin-treated diabetes, acute hypoglycaemia is a very common occurrence. Patients with type 1 diabetes attempting to achieve strict glycaemic control suffer countless episodes of asymptomatic hypoglycaemia; plasma glucose concentrations may be less than 3.3 mmol/l 10% of the time. In humans the most important organ to be affected by fluctuations in blood glucose is the brain. The human brain is dependent upon glucose as its main source of energy and rapidly malfunctions if deprived of this substrate. In order to protect the function and integrity of the central nervous system, blood glucose levels are normally very strictly controlled. It is known that progressive neuroglycopenia causes cognitive impairment and the present studies have demonstrated the profound impact of acute hypoglycaemia on the crucially important cognitive domain of memory. We have also shown that acute *hyper*-glycaemia can have a harmful effect on neuropsychological function and this is naturally of major concern to people with diabetes who, intrinsically, are exposed to higher than normal levels of blood glucose.

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Appendix 1

Abstracts

Sommerfield AJ, McAulay V, Deary IJ, Frier BM.

Short-term, delayed and working memory are impaired during hypoglycaemia in humans with and without type 1 diabetes.

1. Scottish Society for Experimental Medicine, Dundee, UK, 2001
Abstract: Scottish Medical Journal 2002; 47: 16.
2. Diabetes UK Annual Professional Conference, Birmingham, UK, 2002
Abstract: Diabetic Medicine 2002; 19 (Suppl. 2): 21.
3. 20th meeting Anglo Danish Dutch Diabetes Group, Coventry, UK, 2002
4. American Diabetes Association, San Francisco, USA, 2002
Abstract: Diabetes 2002

Sommerfield AJ, Wilkinson IB, Webb DJ, Frier BM.

Central haemodynamic effects of insulin and hypoglycaemia in humans with and without type 1 diabetes.

1. Diabetes UK Annual Professional Conference, Birmingham UK, 2002.
Abstract: Diabetic Medicine 2002; 19 (Suppl. 2): 66.
2. EASD Annual Professional Conference, Budapest, Hungary, 2002.
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Acute hyperglycaemia alters mood state and impairs cognitive performance in people with type 2 diabetes.

1. Diabetes UK Annual Professional Conference, Glasgow UK, 2003.
Abstract: Diabetic Medicine 2003; 20 (Suppl. 2): 31.

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Vessel wall stiffness and the central haemodynamic effects of insulin and hypoglycaemia in humans with and without type 1 diabetes.

1. Scottish Society for Experimental Medicine, Glasgow UK, 2004.
Abstract: Scottish Medical Journal 2004; 49: 18.

Appendix 2

Papers

Sommerfield AJ, Deary IJ, McAulay V, Frier BM.

Moderate hypoglycemia impairs multiple memory functions in healthy adults.

Neuropsychology 2003; **17**: 125-132.

Deary IJ, Sommerfield AJ, McAulay V, Frier BM.

Moderate hypoglycaemia obliterates working memory (Letter).

Journal of Neurology, Neurosurgery and Psychiatry 2003; **74**: 278-279.

Sommerfield AJ, Deary IJ, McAulay V, Frier BM.

Short-term, delayed and working memory are impaired during hypoglycemia in people with type 1 diabetes.

Diabetes Care 2003; **26**: 390-396.

Sommerfield AJ, Deary IJ, Frier BM.

Acute hyperglycemia alters mood state and impairs cognitive performance in people with type 2 diabetes

Diabetes Care 2004; **27**: 2335-2340.

Moderate Hypoglycemia Impairs Multiple Memory Functions in Healthy Adults

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The effects of acute insulin-induced hypoglycemia on short-term, delayed, and working memory were examined in healthy adults. A hyperinsulinemic glucose clamp was used to maintain arterialized blood glucose at either 4.5 (euglycemia) or 2.5 (hypoglycemia) mmol/L on 2 separate occasions in 16 healthy volunteers. Tests of immediate and delayed verbal memory, immediate and delayed visual memory, and working memory were administered during each experimental condition. All memory systems were impaired during acute hypoglycemia, with working memory and delayed memory being particularly susceptible. These findings are informative concerning the metabolic basis of adequate memory function and are of practical importance to people with insulin-treated diabetes, in whom hypoglycemia is common.

Acute hypoglycemia is a common side effect of treatment with insulin in people with diabetes (Tattersall, 1999). The nonphysiological doses of insulin that are used in standard treatment regimens often lead to a mismatch between blood glucose and plasma insulin concentrations, resulting in hypoglycemia. Hypoglycemia can be subdivided into mild (self-treated) and severe episodes. In the latter, external assistance is required for recovery. A recent study has confirmed the high frequency of hypoglycemia among people with Type 1 diabetes (Pramming et al., 2000). The mean rate of mild hypoglycemia was 2.0 episodes/patient/week, and the mean rate of severe hypoglycemia was 1.3 episodes/patient/year. Hypoglycemia is considerably less common in people with Type 2 diabetes. Although hypoglycemia can also be associated with sulphonylurea therapy, this occurs much less frequently than insulin-induced hypoglycemia and is seldom severe.

The human brain is dependent on a continuous supply of glucose as its main source of energy. Cerebral deprivation of glucose, therefore, rapidly causes cognitive dysfunction through the direct effects of acute neuroglycopenia (Deary, 1993, 1998). Early observations noted that blood glucose concentrations below 3.0 mmol/L, as a consequence of either natural glucose fluctuations (Flender & Lifshitz, 1976) or bolus injection of insulin (Russell & Rix-Trot,

1975), were associated with impaired motor coordination and mental speed. Since then, a large literature on the cognitive effects of acute hypoglycemia has developed, with recent interest focusing on the particular cognitive domains that are affected and the clinical relevance of the cognitive decrements that occur.

Two main techniques have been used to induce hypoglycemia experimentally: the insulin infusion technique and the hyperinsulinemic glucose clamp. The former involves the intravenous infusion of insulin at various rates to reach the desired blood glucose concentration, whereas the hyperinsulinemic clamp technique uses a fixed intravenous infusion rate of insulin with a variable intravenous infusion of dextrose to maintain blood glucose concentrations at the determined level (DeFronzo, Tobin, & Andres, 1979). However, in many clamp studies, a common methodological error has been to induce hypoglycemia by glucose clamp using a stepwise decline in blood glucose, with tests of cognitive function being administered during the final 30 min of each blood glucose plateau. This allows a practice effect to occur, which is confounded with the level of hypoglycemia.

Hypoglycemia affords the opportunity to study cognitive processes under controlled deprivation of the brain's energy supply. Tasks that are complex, are attention demanding, and require a rapid response are more impaired during neuroglycopenia, compared with simple cognitive and motor tasks, which are relatively preserved. In general, tests that involve attention, concentration, psychomotor skill, the accessing of long-term memory, and the ability to ignore distracting information tend to deteriorate when blood glucose declines below about 3.0 mmol/L (Hoffman et al., 1989; Kerr, Macdonald, & Tattersall, 1991; Mittraku et al., 1991; Pramming, Thorsteinsson, Theilgaard, Pinner, & Binder, 1986; Widom & Simonson, 1990; Wirsén, Tallroth, Lindgren & Agardh, 1992).

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Memory is one of the most important cognitive domains with respect to everyday function, yet it has seldom been examined in detail during acute hypoglycemia. A still useful classification recognized three main memory systems—sensory, short-term, and long-term memory (Baddeley, 1996). Although the measurement of sensory memory presents practical problems, the effect of hypoglycemia on this memory system has been examined in studies conducted in the Royal Infirmary of Edinburgh (Ewing, Deary, McCrimmon, Strachan, & Frier, 1998; McCrimmon, Deary, & Frier, 1997; McCrimmon, Deary, Huntly, MacLeod, & Frier, 1996). Less is known about the effects of hypoglycemia on other aspects of memory. Short-term memory capacity is limited in terms of the number of items that can be stored, and it lasts for a duration measured in seconds. Rehearsal of information allows material to be transferred from short-term memory to long-term memory by the process of consolidation (Fuster, 1995; Hebb, 1949; Horn, 1998).

Working memory is a short-term memory system that allows the retention and manipulation of information concurrently. Working memory comprises a phonological loop (a store of phonetic, verbal information), a visuospatial scratchpad (a store of spatial information and memories for movement), and a central executive, which supervises and updates the content of working memory (Baddeley, 1986, 1992; Baddeley & Hitch, 1974). Working memory is used, for example, to remember what has been said at the beginning of a sentence and retain this until the sentence has been completed; it allows a telephone number to be remembered long enough for it to be dialed and enables the calculation of simple mental arithmetic. Multivariate modeling and computer simulation studies show that working memory correlates highly with individual differences in the general factor derived from a battery of psychometric intelligence tests (Deary, 2001; Engle, Tuholski, Laughlin, & Conway, 1999; Kyllonen & Christal, 1990; Stauffer, Ree, & Carretta, 1996). To our knowledge, the effect of hypoglycemia on this key cognitive area has not explicitly been studied previously, and there are no systematic studies involving short- and longer term memory processes.

Previous studies of acute hypoglycemia used a heterogeneous assortment of memory measures as part of a larger battery of cognitive tests. Short-term memory, as tested by word and story recall, can deteriorate during hypoglycemia (Draeos et al., 1995; Holmes, Koepke, Thompson, Gyves, & Weydert, 1984; Pramming et al., 1986; Widom & Simonson, 1990; Wirsén et al., 1992). Other tests that assess memory can be identified in cognitive batteries that have been used to study the effects of acute insulin-induced hypoglycemia in subjects with and without diabetes. Some studies have shown impaired memory functions (Draeos et al., 1995; Holmes et al., 1984; Mitrakou et al., 1991; Pramming et al., 1986; Wirsén et al., 1992), whereas, in others, tests of memory were unaffected (Harrad et al., 1985; Holmes, Hayford, Gonzalez, & Weydert, 1983; Widom & Simonson, 1990). Variation in results may relate to methodological differences among studies. Many of the studies reported previously suffer from small sample sizes, application of a limited selection of tests, and an inadequate

ascertainment of biomedical and psychosocial variables that may affect the results. In addition, the method of induction of hypoglycemia has varied among studies, and the method of blood sampling (e.g., arterialized or venous blood) has differed. As a result of these problems, definitive evidence regarding the effects of acute hypoglycemia on learning and memory is not available. There is a particular lack of information concerning the effect of hypoglycemia on working memory.

The present study was designed to investigate the effects of experimentally induced hypoglycemia on verbal and nonverbal tests of short-term, delayed, and working memory in young, healthy humans.

METHOD

Subjects

Sixteen (9 male) healthy adult volunteers were studied. Their mean age was 29.6 years ($SD = 2.9$), and they had a mean body mass index of 24.1 kg/m^2 ($SD = 1.1$). None of the subjects had diabetes or a family history of diabetes, a history of chronic disease, previous head injury, seizure, blackouts, or psychiatric illness. They had no intercurrent illness and were not taking any regular medication (with the exception of the oral contraceptive pill). Ethical permission for the study was approved by the Lothian Research Ethics Committee. All subjects gave written, informed consent for the study.

Study Design

Each subject participated in two laboratory sessions, representing two different experimental conditions, that were separated by at least 2 weeks. The studies were conducted in the Department of Diabetes at the Royal Infirmary of Edinburgh. A modified hyperinsulinemic glucose clamp was used to maintain the blood glucose at a predetermined level. In the euglycemia condition, the arterialized blood glucose concentration was maintained at 4.5 mmol/L and hypoglycemia was not induced. In the hypoglycemia condition, the glucose concentration was lowered to 2.5 mmol/L . The subjects were not informed of which experimental arm of the study was being performed on each occasion, and they underwent the two experimental sessions in a randomized and counterbalanced fashion.

Procedure

Each session commenced at 8 a.m. following a 10–12-hr overnight fast. An intravenous cannula was inserted in a retrograde direction into a vein on the dorsum of the nondominant hand for regular blood sampling. The hand was placed in a heated blanket to arterialize the venous blood. A second intravenous cannula was inserted into a vein in the antecubital fossa of the same arm for infusion of human soluble insulin (Humulin S; Eli Lilly, Indianapolis, IN) and 20% dextrose. Intradermal lignocaine (1%) was used for insertion of each cannula. Insulin was infused at a constant rate of $60 \text{ mU/m}^2/\text{min}$ using an IMED Gemini PC1 pump (Alaris Medical Systems, San Diego, CA). A variable intravenous infusion of 20% dextrose was given simultaneously. The rate of dextrose infusion was varied according to the arterialized blood glucose concentration. This was measured at the bedside using the glucose oxidase method (Yellow Springs Instrument 2300 Stat, Yellow

Springs, OH). Blood glucose concentration was measured every 3 min initially and then every 5 min once a stable level had been achieved.

In each study condition, the arterialized blood glucose concentration was stabilized initially at 4.5 mmol/L (baseline) for a period of 30 min. In the euglycemia condition, the blood glucose concentration was thereafter maintained at 4.5 mmol/L throughout the study. In the hypoglycemia condition, blood glucose was lowered over a 20-min period to 2.5 mmol/L. The blood glucose concentration was maintained at the predetermined target level for a further 10 min before we commenced the cognitive tests and was maintained at this level for a further 70 min while the tests were administered. At the end of the hypoglycemia study, the blood glucose level was restored to 4.5 mmol/L. Subjects were provided with a meal after completion of each study session.

Cognitive Function Tests

Tests of immediate and delayed verbal memory, immediate and delayed visual memory, and working memory were administered during the study conditions. In addition to the memory tests, the Trail Making B Test (Reitan, 1958) and the Digit Symbol Substitution Test (Wechsler, 1998) were also administered. These latter tests were used to confirm the effect of hypoglycemia on cognitive function, as has been shown in previous studies (Ewing et al., 1998; Holmes et al., 1984; McCrimmon et al., 1997; Stevens et al., 1989; Wirsén et al., 1992).

Memory Tests

Verbal Memory Tests

Auditory Verbal Learning Test—Immediate and delayed. The Auditory Verbal Learning Test (AVLT; Lezak, 1995) is a test of immediate memory span, retrieval efficiency, and learning. The delayed component measures longer term retention (Lezak, 1995). It consists of a list of 15 words that are read to the subject at a rate of 1 word per second. The subject is asked to try to recall words immediately in any order from the list. This procedure is repeated five times with the same list. The total of words remembered correctly is designated the *immediate* score. Each subject is instructed to try to remember the words on the list and is informed that recall of words from the list will be requested after a period of 1 hr. The *delayed* score is the number of words that are recalled correctly at that time. The number of words that were remembered (i.e., percentage retained) from the immediate test is also calculated.

Logical Memory Test—Immediate and delayed. The Logical Memory Test (Wechsler, 1987) is a test of verbal learning. It measures immediate free recall following auditory presentation as well as delayed recall. The subject is asked to recount a short story immediately after it is read to him or her, to remember the story, and to recall it again after a delay of 1 hr. The story incorporates a number of specific points or story elements, each of which the subject must recall to obtain credit. The immediate and delayed scores are the sum of the number of points remembered by the subject during immediate and delayed recall, respectively. From these scores, the percentage of information retained from the immediate to the delayed presentation is calculated.

Visual Memory Tests

Visual Reproduction Test—Immediate and delayed. The Visual Reproduction Test (Wechsler, 1987) measures immediate and

delayed recall following nonverbal, visual presentation. A design (line drawing) is presented to the subject. The subject is allowed to study the design for 10 s. The design is then removed, and the subject is instructed to draw the design from memory. The next design follows, and so on for a total of five designs. The subject is asked to draw the designs again after a delay of at least 1 hr. The designs are scored according to their accuracy. The total score is the sum of the scores of all five designs. Immediate and delayed scores are calculated separately, and from these the percentage retained score is also derived.

Benton Visual Retention Test. The Benton Visual Retention Test (BVRT; Sivan, 1992) is a test of immediate visual recall. A series of 10 designs of increasing complexity are presented to the subject, the designs are removed, and the subject is then asked to draw the designs from memory. The test is scored according to the accuracy with which the designs are drawn. The total score is the sum of the scores of all 10 designs.

Working Memory Tests

Working Digit Span Test—Forward and backward. In the Working Digit Span Test (Wechsler, 1987), a series of lists of numbers is presented verbally to the subject. The standard Wechsler Working Digit Span Test was modified for this experiment to more fully test working memory. The subjects were asked to recall the numbers in ascending numerical order (forward) or reverse numerical order (backward). For example, for the sequence 2, 6, 1, 5, 3, the correct response for the Working Digit Span Test—forward is 1, 2, 3, 5, 6, and for the Working Digit Span Test—backward, it is 6, 5, 3, 2, 1. The test score is the number of lists that are remembered correctly.

Letter/Number Sequence Test. In the Wechsler (1987) Letter/Number Sequence Test, a series of lists of numbers mixed with letters is presented verbally. The subject must recall the list, stating the numbers in ascending numerical order followed by the letters in alphabetical order. For example, for the sequence 2, D, 6, A, 1, G, the correct response is 1, 2, 6, A, D, G. The test score is the number of lists that are recounted correctly.

Validation Span Test. This test examined working memory in the context of numerical reasoning and was provided by Kyllönen (1993; Kyllönen & Christal, 1990). This test is based on Baddeley's (1986) original working memory tasks, but the material has been altered to be less abstract. In this test, the subject is presented visually with a simple arithmetical problem. The subject is required to perform the calculation and to determine whether the sum is correct or incorrect. On the left side of the page, adjacent to the problem, is placed an isolated and unrelated number. In addition to performing the simple mental arithmetic, the subject must remember this isolated number. Each problem is presented for 5 s. The problems are presented in sets of three, four, and five. After completion of each set, the subject must recall the isolated left-sided numbers in the correct order. The score is based on how many of the isolated numbers are remembered correctly. An example of a set of three is given below:

5	$3 + 2 - 1 = 4$	(correct)
2	$1 - 5 + 8 = 7$	(false)
3	$2 + 4 + 6 = 12$	(correct)

For this example, the correct sequence of numbers is 5, 2, 3.

Parallel versions of the tests are available for Logical Memory, AVLT, BVRT, and Validation Span, and in the present study these were used to minimize a learning effect between the two study conditions. Throughout the study, the battery of tests was carried out in a fixed order.

Nonmemory Tests

Trail Making B

In the Trail Making B Test (Reitan, 1958), the subject is presented with a grid containing randomly positioned numbers and letters. The subject must connect consecutive numbers in numerical order and consecutive letters in alphabetical order, alternating between numbers and letters. The score is the time taken to complete the task.

Digit Symbol Substitution Test

The Digit Symbol Substitution Test (Wechsler, 1998) consists of four rows containing, in all, 100 small blank squares, each paired with a randomly assigned number from 1 to 9. Above these rows is a printed key that pairs each number with a different symbol. The subject is asked to fill in as many of the blank squares as possible with the appropriate symbol that matches the number above the box, in a time limit of 120 s. The score is the number of squares that are successfully completed within the 120-s time limit.

Symptoms of Hypoglycemia

The Edinburgh Hypoglycemia Scale (Deary, Hepburn, MacLeod, & Frier, 1993), a validated, subjective self-rating questionnaire, was used to document the symptoms of hypoglycemia experienced by the subjects during the two studies. The symptoms of hypoglycemia were classified as autonomic (e.g., hunger, palpitations, sweating, shaking), neuroglycopenic (e.g., drowsiness, confusion, inability to concentrate, speech difficulty, blurred vision), and nonspecific (e.g., nausea, headache). Each symptom was graded on a Likert scale ranging from 1 (*not present*) to 7 (*very intense*).

Statistical Analysis

A general linear model (repeated measures analysis of variance) was used, with order of session (euglycemia–hypoglycemia or hypoglycemia–euglycemia) as a between-subjects factor and condition (euglycemia or hypoglycemia) as a within-subject factor. A *p* value of less than .05 was considered to be significant. Effect size was calculated using eta squared. All analyses were performed using SPSS Version 10.0 for Windows.

RESULTS

A stable blood glucose plateau was achieved during both study conditions. The mean blood glucose concentration during the euglycemia condition was 4.5 mmol/L (*SD* = 0.2), and during the hypoglycemia condition it was 2.5 mmol/L (*SD* = 0.1). Statistical analysis revealed that no significant order effects had occurred for any of the outcome variables of this study, and no significant gender differences were evident on any test.

Symptoms

Results of the hypoglycemia symptom questionnaires confirmed that scores for autonomic, $F(1, 14) = 21.24, p < .0001$, neuroglycopenic, $F(1, 14) = 31.78, p < .0001$, and general malaise, $F(1, 14) = 4.34, p = .05$, symptoms were all significantly higher during hypoglycemia, compared

with baseline euglycemia, and were unchanged from baseline levels during the euglycemia study condition.

Tests of Nonmemory Function

During hypoglycemia, the time taken to complete the Trail Making B Test increased significantly, from a mean of 41.5 s (*SD* = 7.9) during euglycemia to 62.6 s (*SD* = 11.0) during hypoglycemia, $F(1, 14) = 58.5, p < .0001, \eta^2 = .81$. The mean score of the Digit Symbol Substitution Test deteriorated from 70.1 (*SD* = 13.0) during euglycemia to 59.6 (*SD* = 14.4) during hypoglycemia, $F(1, 14) = 19.01, p = .001, \eta^2 = .58$.

Tests of Memory

The results of the memory function tests are summarized in Table 1.

Immediate Verbal Memory

Acute hypoglycemia caused a significant deterioration in tests of immediate verbal memory as assessed by the AVLT, $F(1, 14) = 16.12, p = .001, \eta^2 = .54$, and Logical Memory Test, $F(1, 14) = 11.57, p = .004, \eta^2 = .45$.

Immediate Visual Memory

The BVRT score declined during hypoglycemia, $F(1, 14) = 5.09, p = .041, \eta^2 = .27$. By contrast, the results for the Visual Reproduction Test did not demonstrate a significant deterioration, $F(1, 14) = 4.23, p = .059$.

Delayed Verbal Memory

Performance in both the AVLT–delayed, $F(1, 14) = 96.18, p < .0001, \eta^2 = .87$ (percentage retained, $F(1, 14) = 53.65, p < .0001, \eta^2 = .79$), and the Logical Memory Test–delayed, $F(1, 14) = 19.04, p = .001, \eta^2 = .58$ (percentage retained, $F(1, 14) = 37.31, p < .0001, \eta^2 = .73$), was significantly impaired during hypoglycemia.

Delayed Visual Memory

The Visual Reproduction Test–delayed demonstrated a significant decrement during hypoglycemia, $F(1, 14) = 18.96, p = .001, \eta^2 = .58$ (percentage retained, $F(1, 14) = 12.49, p = .003, \eta^2 = .47$).

Working Memory

All working memory test scores deteriorated significantly during hypoglycemia: Working Digit Span–forward, $F(1, 14) = 5.00, p = .042, \eta^2 = .26$; Working Digit Span–backward, $F(1, 14) = 28.90, p < .0001, \eta^2 = .67$; Letter/Number Sequencing, $F(1, 14) = 9.44, p = .008, \eta^2 = .40$; Validation Span, $F(1, 14) = 225.36, p < .0001, \eta^2 = .94$. Validation Span deteriorated from a mean score of 22.0 (*SD* = 2.3) during euglycemia to 13.4 (*SD* = 2.5) during hypoglycemia.

Table 1
Results of Tests of Memory Function During Euglycemia and Hypoglycemia in 16 Healthy Adults

Memory system and subtest	Euglycemia		Hypoglycemia		<i>p</i>	η^2
	<i>M</i>	<i>SD</i>	<i>M</i>	<i>SD</i>		
Immediate verbal memory						
Immediate Logical Memory	27.7	5.9	24.4	5.0	.004	.45
Immediate AVL T	43.6	7.0	33.9	7.7	.001	.53
Immediate visual memory						
Benton Visual Retention test	6.3	1.7	5.1	1.6	.041	.26
Visual reproduction	85.6	8.3	81.7	8.0	.059	.23
Delayed memory						
Delayed Logical Memory	15.1	4.0	8.1	3.9	.001	.58
% retained	88.2	10.0	55.6	19.7	<.0001	.73
Delayed AVL T	10.3	2.1	4.5	1.6	<.0001	.87
% retained	88.2	8.9	50.6	18.3	<.0001	.79
Delayed visual reproduction	21.5	16.5	7.6	5.5	.001	.57
% retained	25.9	20.3	10.0	7.3	.003	.47
Working memory						
Validation span	22.0	2.3	13.4	2.5	<.0001	.94
Working Digit Span forward	10.2	2.4	9.3	1.7	.042	.26
Working Digit Span backward	8.8	1.9	6.8	1.2	<.0001	.67
Letter/Number Sequence	13.1	3.1	10.5	1.8	.008	.40

Note. AVL T = Auditory Verbal Learning Test.

DISCUSSION

The present study has demonstrated that acute hypoglycemia significantly impaired multiple memory functions in young, healthy humans. The results indicate that all of the memory systems tested were impaired during hypoglycemia, with working memory and delayed memory being particularly susceptible. The effect sizes (eta squared) for working memory and delayed memory were large; the effect of moderate hypoglycemia on some of these processes was profound. Performance in the Trail Making B and Digit Symbol Substitution Tests was also significantly impaired during hypoglycemia, consistent with the results of previous studies (Ewing et al., 1998; Holmes et al., 1984; McCrimmon et al., 1997; Stevens et al., 1989; Wirsén et al., 1992), confirming that the degree of hypoglycemia achieved was sufficient to impair other domains of cognitive function simultaneously.

Few studies have examined in detail the effects of acute hypoglycemia on memory function in humans with and without diabetes (Draeos et al., 1995; Holmes et al., 1983, 1984; Widom & Simonson, 1990; Wirsén et al., 1992), and earlier studies have given inconsistent results. In view of the paucity of data on the effects of hypoglycemia on this important cognitive domain, the present study has attempted a broad examination of the effects of hypoglycemia on short-term, delayed (longer term), and working memory using verbal and nonverbal materials.

The present study has shown that short-term memory is significantly disrupted by hypoglycemia. The effect of hypoglycemia on short-term verbal memory, assessed by the AVL T and Logical Memory Test, was considerable. Performance in both tests was significantly impaired, consistent with the results of earlier studies (Draeos et al., 1995; Wirsén et al., 1992). In the present study, the extent of the

deterioration induced by hypoglycemia was substantial, as indicated by the large effect size. The extent of deterioration appeared to be slightly greater in the AVL T compared with the Logical Memory Test. The capacity of short-term memory to retain verbal material depends on how much meaning the information confers to the recipient. A list of random, unrelated words is therefore more difficult to remember than a story in which the words are organized into meaningful sentences with a structured and logical progression. Short-term visual memory was also impaired during hypoglycemia. The BVRT score decreased significantly during hypoglycemia, although this test was impaired to a lesser degree than were tests of verbal memory. This may reflect an integral difference in the degree of susceptibility to hypoglycemia of these two different modalities, or it may indicate a relative insensitivity of this specific test in detecting changes in cognitive performance. The Visual Reproduction Test—immediate was not significantly affected by hypoglycemia. Although a small decrement was observed in the scores during hypoglycemia as compared with euglycemia, this difference was not significant.

All tests of delayed memory were greatly affected by hypoglycemia. Performance in the AVL T—delayed and the Logical Memory Test—delayed decreased substantially, with the AVL T—delayed appearing to be more vulnerable to the effects of hypoglycemia. A significant deterioration in performance in tests of delayed verbal memory has been shown in earlier studies of nondiabetic (Mitrakou et al., 1991) and diabetic humans (Fanelli et al., 1998). In the present study, the effects of hypoglycemia on delayed visual memory were also considerable. Analysis of the immediate and delayed Logical Memory Test, AVL T, and Visual Reproduction Test scores allowed calculation of the percentage of information that was learned and retained during the

study. This revealed that, for tests of both verbal and visual memory, not only did subjects recall less information immediately after having studied the information during hypoglycemia compared with euglycemia, over time, they forgot more of what they had recalled immediately during hypoglycemia than during the euglycemic state.

Working memory was very susceptible to the effects of hypoglycemia, with all tests being affected severely. The Working Digit Span Test—forward, the easiest of the working memory tests, was the least affected. Working Digit Span—backward, Letter/Number Sequencing, and Validation Span were all impaired more substantially. The impact of hypoglycemia on performance in the Validation Span Test, in particular, was highly significant, and the size of the decrement, as measured by effect size, was very large, with the glycemic manipulation accounting for almost all of the variance in the test scores.

In the brain, the medial temporal lobe and, in particular, the hippocampus are the principal structures involved with memory performance (Gabrielli, Brewer, Desmond, & Glover, 1997; Parkin, 1996; Scoville & Milner, 1957; Stern et al., 1996). Neuropathological observations have indicated that the brain is susceptible to neuroglycopenia in a rostro-caudal direction, with the cerebral cortex and hippocampus being most sensitive and the brainstem and spinal cord being most resistant (Auer, Wieloch, Olsson & Siesjo, 1984). The brains of human subjects who have suffered an episode of severe hypoglycemia have been studied and are shown to have areas of cortical necrosis in the frontal lobes and hippocampus, with relative sparing of the hindbrain (Auer et al., 1984; Fujioka et al., 1997). Anecdotal case reports of severe amnesia following an episode of severe hypoglycemia have identified a specific structural lesion in the hippocampus in people with insulin-treated diabetes (Chalmers et al., 1991; Holemans, Dupuis, Misson & Vanderijst, 2001). The hippocampus, therefore, may be particularly vulnerable to the adverse effects of hypoglycemia.

This premise is supported by the results of the present study, which clearly demonstrate the detrimental effects of short-term acute hypoglycemia on memory. Working memory and delayed memory appear to be the components of memory most vulnerable to the induction of neuroglycopenia. Functional magnetic resonance imaging studies have shown that prefrontal brain activity is associated with the performance of tasks very similar to those performed in working memory tests (Carpenter, Just, & Reichle, 2000; Fletcher & Henson, 2001; McIntosh, 1999). In addition, many of the tests of working memory also engage other cognitive processes, particularly attention, that also use the frontal lobe (Cohen et al., 1988; Pardo, Fox, & Raichle, 1991). These areas of the brain are also very sensitive to the effects of neuroglycopenia (Auer et al., 1984; Pramming et al., 1986), and a study in the Royal Infirmary of Edinburgh has confirmed the detrimental effects of acute hypoglycemia on attention (McAulay, Deary, Ferguson, & Frier, 2001). In the present study, it is possible that the disruptive effect of hypoglycemia on working memory was enhanced by the simultaneous adverse effect of hypoglycemia on attention.

In the brain, glucose is transported across the cell membranes by facilitated diffusion, mediated by the glucose transport proteins GLUT1 and GLUT3 (Duelli & Kuschinsky, 2001). These are localized in the membranes of brain endothelial cells, astrocytes, and neurones. In animals, working memory tasks have been shown to exert high demands on brain extracellular glucose (McNay, Fries, & Gold, 2000), and it is possible that the vulnerability of working memory to the effects of hypoglycemia is a consequence of the interaction between local energy demands of the brain and the regional distribution of GLUT1 and GLUT3 receptors. The profound impact of hypoglycemia on delayed memory is likely to be a consequence of the disruption, by neuroglycopenia, of the normal pathways of hippocampal neural activity that are responsible for the transfer of information from short-term to longer term memory.

In summary, the present study is novel in examining three major aspects of memory for both verbal and nonverbal stimuli. In particular, it has systematically studied and unequivocally demonstrated the extent to which working memory processes are affected by moderate hypoglycemia, and it forms a foundation for research that examines the biological and practical aspects of these findings. It also lays a foundation for more psychological research, which should inquire, for example, whether the processes of consolidating (laying down) new memories or retrieving already-established memories are particularly sensitive to the effects of hypoglycemia. The present study also demonstrates that other cognitive processes are impaired during moderately severe hypoglycemia. It could be argued that the impairment of memory performance is a reflection of a diffusely distributed transient brain dysfunction induced by hypoglycemia and that the very large effect size associated with the Validation Span Test, in particular, is a consequence of task complexity rather than specific vulnerability of memory processes to hypoglycemia.

In addition to contributing to knowledge about the biological bases of intact memory function, the present results have practical importance. Working memory shows a strong connection to fluid intelligence (Engle et al., 1999; Kyllonen & Christal, 1990; Stauffer et al., 1996) and is necessary for performance of such complex cognitive tasks as language comprehension and reasoning (Baddeley, 1992; Carpenter, Just, & Shell, 1990; Kyllonen, 1993). Many tasks in everyday life engage memory processes, especially working memory. For example, working memory is used to remember whether traffic is proceeding along a street after one looks to either side before crossing, is used in making rapid decisions while one is driving or operating machinery or computer equipment, and is fundamental to following instructions or reading a map. People with insulin-treated diabetes are frequently exposed to hypoglycemia as a side effect of insulin therapy. These individuals may be subject to impairments of multiple memory functions during hypoglycemia. This may have important practical implications for daily activities and working ability. The detrimental effect of hypoglycemia on delayed memory also suggests that the ability to learn during hypoglycemia could be

substantially impaired and could influence academic performance, such as during examinations. Whether these findings are of practical importance to people with insulin-treated diabetes remains to be established. It is possible that people with diabetes may develop compensatory mechanisms to enable them to cope with the adverse effects of hypoglycemia. Further studies are currently investigating the effects of hypoglycemia on memory in people with Type 1 (insulin-dependent) diabetes.

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Short-Term, Delayed, and Working Memory Are Impaired During Hypoglycemia in Individuals With Type 1 Diabetes

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OBJECTIVE — To examine the effects of acute insulin-induced hypoglycemia on short-term, delayed, and working memory in individuals with type 1 diabetes.

RESEARCH DESIGN AND METHODS — A hyperinsulinemic glucose clamp was used to maintain arterialized blood glucose level at either 4.5 mmol/l (euglycemia) or 2.5 mmol/l (hypoglycemia) on two separate occasions in 16 adults with type 1 diabetes. The participants completed tests of immediate and delayed verbal memory, immediate and delayed visual memory, and working memory during each experimental condition. Two other mental tests, the Trail Making B Test and the Digit Symbol Test, were also administered.

RESULTS — Performance in tests of immediate verbal and immediate visual memory was significantly impaired during hypoglycemia. The effect of hypoglycemia on working memory and delayed memory was more profound. Performance in the nonmemory tests, the Trail Making B Test, and the Digit Symbol Test also deteriorated during hypoglycemia.

CONCLUSIONS — All of the memory systems examined in the present study were affected significantly by acute hypoglycemia, particularly working memory and delayed memory. Mild (self-treated) hypoglycemia is common in individuals with insulin-treated diabetes; therefore, these observed effects of hypoglycemia on memory are of potential clinical importance because they could interfere with many everyday activities.

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Hypoglycemia is a common adverse effect of treatment with insulin in individuals with diabetes (1,2). Acute neuroglycopenia causes rapid deterioration of cognitive function in humans with and without diabetes (3). Complex and attention-demanding tasks, as well as those that require a rapid response, are more affected by neuroglycopenia, whereas simple motor and cognitive tasks are relatively preserved. In general, results of tests that involve attention, concentration, psychomotor skills, accessing of

long-term memory, and ability to ignore distracting information deteriorate when arterial blood glucose level declines below ~3.0 mmol/l (4–9).

Memory is one of the most important cognitive domains with respect to everyday function and is the process of storing, encoding, and retrieving information. Different forms of memory are recognized, including sensory, short-term, long-term, and working memory (10). In sensory memory, representations of the physical features of a stimulus are stored

for a very brief time (≤ 1 s), and it is difficult to distinguish from the process of perception. The deleterious effect of hypoglycemia on this memory system has been demonstrated in previous studies conducted in our center (11–14). It seems that the principal function of sensory memory is to retain information for a period of time sufficient to allow its transfer to short-term memory. Short-term memory refers to the function that temporarily retains stimuli that have just been perceived. Its capacity is limited in terms of the number of items that can be stored and lasts for ~20 s. Through repetition, information may be transferred from short-term memory to long-term memory. Long-term memory refers to information that is represented on a more permanent basis. Unlike short-term memory, long-term memory has no known limits to capacity and is relatively durable.

Working memory is a short-term memory system that allows concurrent retention and manipulation of information (15). It is used for thinking about what is already known and for deriving conclusions on the basis of that knowledge; therefore, working memory is fundamental to successful completion of many activities. For example, it is used to remember what has been said at the beginning of a sentence and retain this until the sentence has been completed and is essential for the calculation of mental arithmetic. It allows spatial relations to be updated in our mental map as we move through a new geographical location.

Few studies have examined the effects of acute hypoglycemia on memory function, other than by including a heterogeneous assortment of memory measures as part of a larger battery of cognitive tests. In some studies (6,7,9,16,17), memory functions were impaired during acute hypoglycemia, whereas in other studies (8,18,19), memory was apparently unaffected. Variability of results may relate to

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Abbreviations: AVLT, Auditory Verbal Learning Test; BVRT, Benton Visual Retention Test.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

methodological differences. Many studies are limited by small sample size, application of a limited selection of tests, and inadequate ascertainment of biomedical and psychosocial variables that could affect the results. The method of induction of hypoglycemia has also varied between studies, with differing methods of blood sampling (e.g., arterialized or venous blood). A common methodological error has been induction of a stepwise decrease in blood glucose level with estimation of cognitive function during the final 30 min of each blood glucose plateau, thus confounding glucose level with practice effects. In addition, in a study by Holmes et al. (18), the blood glucose nadir was only 3.3 mmol/l, and the studies by Widom and Simonson (8) and Harrad et al. (19) used only a single test to assess memory function. These factors may also help explain the discrepancy in results between different studies.

In our laboratory, a more comprehensive examination of the effects of moderate acute hypoglycemia on memory performance in healthy nondiabetic humans demonstrated impairment of all memory systems; working memory and delayed memory were most susceptible to the effects of neuroglycopenia (20). The present study was designed to investigate the effects of experimentally induced hypoglycemia on verbal and nonverbal tests of short-term, delayed, and working memory in adults with type 1 diabetes, many of whom frequently have mild (self-treated) hypoglycemia.

RESEARCH DESIGN AND METHODS

Subjects

A total of 16 adults with type 1 diabetes (9 men, 7 women) were studied. Subjects were recruited from the diabetes outpatient clinics at the Royal Infirmary of Edinburgh. The median (range) data for the study participants were as follows: age 28.5 years (20.0–38.2), BMI 23.9 kg/m² (20.1–24.8), duration of diabetes 4.5 years (1.2–8.4), HbA_{1c} 8.2% (6.9–8.7), and insulin dose 0.65 U/kg (0.25–1.1). HbA_{1c} was measured by high-performance liquid chromatography (Variant II Hemoglobin Testing System; Biorad Diagnostics, Hercules, CA) (nondiabetic reference range 4.3–6.5%). None of the participants had a history of hypertension

or chronic disease, previous head injury, seizure, blackouts, alcohol or drug abuse, or psychiatric illness. They had no intercurrent illness and were not taking any regular medication (except for insulin and oral contraceptives). Subjects were excluded from the study if they had any evidence of microvascular disease. Presence of retinopathy was ascertained by ophthalmoscopy, neuropathy was determined by clinical examination, and nephropathy was defined by presence of microalbuminuria. Subjects were excluded if they had a history of impaired awareness of hypoglycemia or had suffered an episode of hypoglycemia in the 48 h preceding the study. All subjects gave written informed consent for participation in the study.

Study design

Each subject participated in two laboratory sessions that were separated by at least 2 weeks. The study was conducted in the Department of Diabetes at the Royal Infirmary of Edinburgh. A modified hyperinsulinemic glucose clamp (21) was used to maintain blood glucose at a predetermined level. In the euglycemia condition, the arterialized blood glucose concentration was maintained at 4.5 mmol/l and hypoglycemia was not induced. In the hypoglycemia condition, the glucose concentration was decreased to 2.5 mmol/l. The subjects were not informed which experimental condition of the study was being undertaken on each occasion, and the two experimental sessions were performed in a randomized and counterbalanced fashion.

Procedure

Each session commenced at 8:00 A.M. after a 10- to 12-h overnight fast, and the subjects omitted their morning dose of insulin. An intravenous cannula for regular blood sampling was inserted retrogradely into a vein on the dorsum of the non-dominant hand, which was placed in a heated blanket to arterialize the venous blood. A second intravenous cannula was inserted into a vein in the antecubital fossa of the same arm for infusion of human soluble insulin (Humulin S; Eli Lilly, Indianapolis, IN) and 20% dextrose. Intradermal lidocaine (1%) was used for insertion of the cannulae. Insulin was infused at a constant rate of 60 mU · m⁻² · min⁻¹ using an IMED Gemini PCI pump (Alaris Medical Systems, San Diego, CA).

A variable intravenous infusion of 20% dextrose was given simultaneously. The rate of dextrose infusion was varied according to the arterialized blood glucose concentration, which was measured at the bedside using the glucose oxidase method (2300 Stat; Yellow Springs Instrument, Yellow Springs, OH). Blood glucose concentration was measured every 3 min until a stable level was achieved and then at 5-min intervals.

In each study condition, the arterialized blood glucose concentration was stabilized initially at 4.5 mmol/l (baseline) for a period of 30 min. In the euglycemia condition, the blood glucose concentration was maintained thereafter at 4.5 mmol/l throughout the study. In the hypoglycemia condition, blood glucose was lowered over a 20-min period to 2.5 mmol/l. The blood glucose concentration was maintained at the predetermined target level for an additional 10 min before commencement of the cognitive tests and was maintained at this level for an additional 70 min during administration of the tests. At the end of the hypoglycemia condition, the blood glucose level was restored to 4.5 mmol/l. Subjects were given a meal after completion of each study.

Cognitive function tests

Tests of immediate and delayed verbal memory, immediate and delayed visual memory, and tests of working memory were administered during the study conditions. In addition to the memory tests, the Trail Making B Test and the Digit Symbol Test were also administered. These tests were used to confirm the effect of hypoglycemia on cognitive function, as has been shown in previous studies (9,11,12,17,22).

Verbal memory tests

Auditory Verbal Learning Test: immediate and delayed. The Auditory Verbal Learning Test (AVLT) is a test of immediate memory capacity, retrieval efficiency, and learning. The delayed component measures longer-term retention (23). It consists of a list of 15 words that are read to the subject at a rate of one word per second. The subject is asked to try to recall words immediately in any order from the list. This procedure is repeated five times. The total of words remembered correctly is designated as the "immediate" score. Each subject is instructed to try to remember the words on the list and is

informed that recall of words from the list will be requested after a period of 1 h. The "delayed" score is the number of words that are recalled correctly at that time. The percentage of words that were retained from the immediate to the delayed score ("percent retained") was also calculated.

Logical Memory Test: immediate and delayed. The Logical Memory Test, a subtest from the Wechsler Memory Scales (24), is a test of verbal learning. It measures immediate free recall after auditory presentation and delayed recall. The subject is asked to recount a short story immediately after it is read to them, to remember the story, and to recall it again after a 1-h delay. The story incorporates 25 specific points or "story elements," each of which the subject must recall to obtain credit. The immediate and delayed scores are the sum of the number of points remembered by the subject during immediate and delayed recall, respectively. From these scores, the percentage of information retained from the immediate to the delayed presentation was also calculated.

Visual memory tests

Visual Reproduction Test: immediate and delayed. This Wechsler Memory Scales subtest measures immediate and delayed recall after nonverbal, visual presentation (24). A design (line drawing) is presented to the subject, who is allowed to study the design for 10 s. The design is then removed and the subject is instructed to draw the design from memory. The next design follows, for a total of five. The subject is asked to draw the designs again after a delay of at least 1 h. The designs are scored according to their accuracy. The total score is the sum of the scores of all five designs. Immediate and delayed scores are calculated separately, and from these the percent retained score was also derived.

Benton Visual Retention Test. The Benton Visual Retention Test (BVRT) is a test of immediate visual recall. A series of 10 designs of increasing complexity are presented to the subject, the designs are removed, and the subject is then asked to draw the designs from memory (25). The test is scored according to the accuracy with which the designs are drawn. The total score is the sum of the scores of all 10 designs.

Working memory tests

Working Digit Span Test: forward and backward. In the digit span test, a series of lists of numbers is presented verbally to the subject. The standard Wechsler Memory Scales Digit Span Test (24) was modified for this experiment to test working memory more fully. The subjects were asked to recall the numbers in ascending numerical order (forward) or reverse numerical order (backward). For example, for the sequence 2-6-1-5-3, the correct response for working digit span forward is 1-2-3-5-6, and for working digit span backward, the correct response is 6-5-3-2-1. The test score is the number of lists that are remembered correctly.

Letter/Number Sequencing Test. In the Letter/Number Sequencing Test from the Wechsler Memory Scales, a series of lists of numbers mixed with letters is presented verbally (24). The subject must recall the list, stating the numbers in ascending numerical order followed by the letters in alphabetical order. For example, for the sequence 2-D-6-A-1-G, the correct response is 1-2-6 A-D-G. The test score is the number of lists that are remembered correctly.

Validation Span Test. In the Validation Span Test from the Kyllonen's Cognitive Abilities Measurement battery (26), the subject is presented visually with a simple arithmetical problem. The subject is required to perform the calculation and to determine whether the sum is correct or incorrect. On the left side of the page, adjacent to the problem, an isolated and unrelated number is placed. In addition to performing the simple mental arithmetic, the subject must remember this isolated number. Each problem is presented for 5 s. The problems are presented in sets of three, four, and five. After completion of each set, the subject must recall the isolated left-sided numbers in the correct order. The score is based on how many of the isolated numbers are remembered correctly. An example of a set of three is given below:

- | | |
|---|-----------------------------|
| 5 | $3 + 2 - 1 = 4$ (correct) |
| 2 | $1 - 5 + 8 = 7$ (incorrect) |
| 3 | $2 + 4 + 6 = 12$ (correct) |

For this example, the correct sequence of numbers is 5-2-3.

Parallel versions of the Logical Memory Test, AVLTL, BVRT, and Validation

Span Test are available; in the present study, these were used to minimize a learning effect between the two study conditions. Throughout the study, the battery of tests was performed in a fixed order.

Other mental tests

Trail Making B Test. The Trail Making B Test from the Halstead Reitan battery (27) assesses complex visual scanning and has a motor component that measures visual conceptual and visual motor tracking. An electronic version of the test, performed on a handheld computer, was used for this study (28). In this test, the subject is presented with a grid containing randomly positioned numbers and letters. The subject is to connect consecutive numbers in numerical order and consecutive letters in alphabetical order, alternating between numbers and letters. The score is the time taken to complete the task.

Digit Symbol Test. This Digit Symbol Test from the Wechsler Adult Intelligence Scale is a test of coding performed at speed. It consists of four rows containing at total of 100 small blank squares, each paired with a randomly assigned number from 1 to 9. Above these rows, a printed key is shown, which pairs each number with a different symbol. The subject is asked to fill in as many of the blank squares with the appropriate symbol that matches the number above the box, in a time limit of 120 s (29). The score is the number of squares that are successfully completed within 120 s.

Symptoms of hypoglycemia

The Edinburgh Hypoglycemia Scale (30), a validated subjective self-rating questionnaire, was used to document the common symptoms of hypoglycemia experienced by the subjects during the two studies. The symptoms of hypoglycemia were classified as autonomic (hunger, palpitations, sweating, shaking), neuroglycopenic (drowsiness, confusion, inability to concentrate, speech difficulty, blurred vision), and nonspecific (nausea, headache). Each symptom was graded using a Likert Scale of 1 (not present) to 7 (very intense).

Statistical analysis

The results were analyzed independently for each memory test. A general linear model (repeated-measures ANOVA) was

Table 1—Results of tests of memory function during euglycemia and hypoglycemia in 16 adults with type 1 diabetes

Memory system	Subtest	Euglycemia	Hypoglycemia	P value	η^2
Immediate verbal memory	Immediate Logical Memory Test	26.8 \pm 4.8	21.3 \pm 6.9	0.008	0.41
	Immediate AVLT	39.4 \pm 7.7	34.0 \pm 5.1	0.002	0.49
Immediate visual memory	Benton Visual Retention Test	6.5 \pm 1.7	4.7 \pm 1.5	0.007	0.42
	Visual Reproduction Test	81.3 \pm 7.2	78.2 \pm 9.5	0.093	0.19
Delayed memory	Delayed Logical Memory Test	13.6 \pm 2.1	6.1 \pm 3.7	<0.0001	0.83
	Percent retained	78.4 \pm 6.0	47.0 \pm 21.3	<0.0001	0.73
	Delayed AVLT	9.1 \pm 1.9	5.3 \pm 1.9	<0.0001	0.68
	Percent retained	79.1 \pm 6.9	47.8 \pm 16.0	<0.0001	0.78
	Delayed Visual Reproduction	15.9 \pm 8.7	7.1 \pm 7.3	0.002	0.52
	Percent retained	18.6 \pm 9.8	9.1 \pm 9.0	0.004	0.46
Working memory	Validation Span Test	20.7 \pm 2.3	14.9 \pm 2.2	<0.0001	0.92
	Modified Digit Span Forward Test	9.56 \pm 2.4	8.6 \pm 1.4	0.06	0.23
	Modified Digit Span Backward Test	8.3 \pm 1.9	7.2 \pm 1.2	0.02	0.33
	Letter/Number Sequencing Test	11.8 \pm 1.9	9.7 \pm 1.6	0.0001	0.57

Data are means \pm SD.

used; order of session (euglycemia-hypoglycemia or hypoglycemia-euglycemia) was a “between subjects” factor, and condition (euglycemia or hypoglycemia) was a “within subjects” factor. A *P* value <0.05 was considered significant. Effect size was calculated using η^2 . An η^2 score of 0.25–0.5 was considered a moderate effect size. All analyses were performed using SPSS statistical software (version 10.0 for Windows; SPSS, Chicago, IL).

RESULTS—A stable blood glucose plateau was achieved during each study condition. The mean (SD) arterialized blood glucose concentration during the euglycemia condition was 4.55 mmol/l (0.18) and during the hypoglycemia condition was 2.51 mmol/l (0.08). Statistical analysis showed that no significant order effects had occurred for any of the outcome variables of this study and no significant sex differences were evident.

Symptoms

Results of the hypoglycemia symptom questionnaires confirmed that scores for autonomic (*P* < 0.0001), neuroglycopenic (*P* = 0.001), and general malaise (*P* = 0.008) symptoms were all significantly elevated during hypoglycemia compared with baseline euglycemia and were unchanged from baseline levels during the euglycemia study condition.

Digit Symbol and Trail Making B Tests

During hypoglycemia, the time taken to complete the Trail Making B Test increased significantly from a mean (SD) of 33.7 s (7.7) during euglycemia to 54.0 s (10.7) during hypoglycemia (*P* < 0.0001, η^2 = 0.68). The mean (SD) score of the Digit Symbol Test declined from 73.5 (11.2) during euglycemia to 62.9 (16.9) during hypoglycemia (*P* = 0.001, η^2 = 0.57).

Tests of memory

The results of the memory function tests are summarized in Table 1.

Immediate verbal memory. Acute hypoglycemia caused a significant deterioration in immediate verbal memory as assessed by the AVLT (*P* = 0.002, η^2 = 0.49) and the Logical Memory Test (*P* = 0.008, η^2 = 0.41).

Immediate visual memory. The BVRT score decreased during hypoglycemia (*P* = 0.007, η^2 = 0.42). By contrast, the score for the visual reproduction test did not decrease significantly (*P* = 0.093).

Delayed verbal memory. Scores for both the Delayed AVLT (*P* < 0.0001, η^2 = 0.68; percent retained, *P* < 0.0001, η^2 = 0.78) and the Delayed Logical Memory Test (*P* < 0.0001, η^2 = 0.83; percent retained, *P* < 0.0001, η^2 = 0.73) were significantly worse during hypoglycemia.

Delayed visual memory. During hypoglycemia, a significant decrement was observed in the Delayed Visual Reproduction Test (*P* = 0.002, η^2 = 0.52; percent retained, *P* = 0.004, η^2 = 0.46).

Working memory. The Working Digit Span Backward Test (*P* = 0.02, η^2 = 0.33), Letter/Number Sequencing Test (*P* = 0.001, η^2 = 0.57), and Validation Span Test (*P* < 0.0001, η^2 = 0.92) all demonstrated a significant decrement during hypoglycemia. The Validation Span Test decreased from a mean (SD) score of 20.7 (2.3) during euglycemia to 14.9 (2.2) during hypoglycemia. However, performance in the Working Digit Span Forward Test was not significantly affected (*P* = 0.06, η^2 = 0.23).

CONCLUSIONS—Few previous studies have made a detailed examination of the effects of acute hypoglycemia on memory function in humans, with and without diabetes (8,9,16–18), and earlier studies have given inconsistent results. Using an identical study design, we have studied the effects of hypoglycemia in young, healthy, nondiabetic volunteers and demonstrated a profound impairment in memory function at a blood glucose level that was similar to the present study (2.5 mmol/l) (20). In view of the frequency with which mild hypoglycemia is experienced by individuals with insulin-treated diabetes, the present study

has examined the effects of acute hypoglycemia on a wide range of memory functions in a group of adults with type 1 diabetes who had no evidence of significant vascular complications.

The present study has demonstrated that in adults with type 1 diabetes, acute moderate hypoglycemia caused a marked deterioration in performance in tests of short-term, delayed, and working memory, for both verbal and nonverbal material; working memory and delayed memory were most strongly affected (according to analysis of effect size). In addition, performance in the Trail Making B Test and the Digit Symbol Test was impaired significantly during hypoglycemia. This is consistent with previous observations (9,11,12,17,22) and confirms that the degree of hypoglycemia achieved was sufficient to affect other domains of cognitive function.

Short-term memory was significantly disrupted by hypoglycemia in the present study. The effect of hypoglycemia on short-term verbal memory, assessed by the AVLT and the Logical Memory Test, and short-term visual memory, assessed by the BVRT, was profound; performance on all three tests was significantly impaired. Earlier studies (9,16) have demonstrated decrements in short-term verbal memory during hypoglycemia. In the present study, the magnitude of the impairment induced by hypoglycemia was substantial, as indicated by the large effect size. However, the immediate Visual Reproduction Test was not significantly affected by hypoglycemia. This seemed to be a manifestation of a "ceiling effect" (i.e., most subjects obtained near-to-perfect scores), indicating a lack of sensitivity of this test to the stress of hypoglycemia.

A significant deterioration in performance on tests of delayed verbal memory has been shown during hypoglycemia in previous human studies of nondiabetic (6) and diabetic subjects (31). In the present study, all tests of delayed memory were markedly affected by hypoglycemia. Performance on the Delayed AVLT and Delayed Logical Memory Tests deteriorated significantly during hypoglycemia, and the effects of hypoglycemia on delayed visual memory were also considerable. Analysis of the immediate and delayed Logical Memory Test, AVLT, and the Visual Reproduction Test scores allowed calculation of the percentage of in-

formation that was learned and retained during the study. This revealed that, for tests of both verbal and visual memory, not only was less information recalled immediately after having studied the information during hypoglycemia as compared with euglycemia, but over time, subjects forgot more of what they had recalled immediately during hypoglycemia than during euglycemia.

Working memory was also very susceptible to the effects of hypoglycemia. The Working Digit Span Forward Test, the least difficult of the working memory tests, was least affected and was not significantly impaired during hypoglycemia. A small decrement was observed in the scores during hypoglycemia as compared with euglycemia, and the effect size was modest, indicating that although the effects of hypoglycemia on this test did not achieve significance in this study, hypoglycemia did impair test performance. The Working Digit Span Backward Test, the Letter/Number Sequencing Test, and the Validation Span Test were all more substantially impaired. The impact of hypoglycemia on performance in these tests was highly significant, and the extent of the decrements, as measured by the effect size, was large.

The adverse effects of hypoglycemia on impairment of memory in adults with type 1 diabetes that have been demonstrated in the present study are very similar to the effects of hypoglycemia on memory function in nondiabetic humans that we have shown previously (20). A previous study (9) has suggested that individuals with diabetes may be more susceptible to cognitive impairment during hypoglycemia. However, comparison of the results of the nondiabetic and diabetic groups showed no significant differences between the two groups. This may be attributable to the fact that the subjects with diabetes in our study were all young, had diabetes of short duration, and had normal hypoglycemia awareness. In addition, subjects in the diabetic and nondiabetic groups were matched for cognitive function. It is not known whether the effect of hypoglycemia on memory is sustained or protracted, and this will require further investigation. Many aspects of cognitive function have been observed to return to normal within 2 h after euglycemia is restored (32–34), and this is presumably similar for memory function.

Functional neuroimaging studies in

humans (35,36) have confirmed that medial temporal lobe structures such as the hippocampus and adjacent parahippocampal regions are the principal structures involved with memory performance. The hippocampus is susceptible to a variety of toxic insults, including heavy metals (37), hypoxia (38), and drugs (39,40). There is some evidence that this particular area of the brain is also preferentially vulnerable to the adverse effects of hypoglycemia. Neuropathological observations have indicated that the brain is sensitive to neuroglycopenia in a rostrocaudal direction and that the cerebral cortex and hippocampus are most susceptible, whereas the brainstem and spinal cord are most resistant (41). Brains of human subjects who have suffered an episode of severe hypoglycemia have been studied (41,42) and were shown to have areas of cortical necrosis in the frontal lobes and hippocampus as well as relative sparing of the hindbrain. Anecdotal case reports of severe amnesia after an episode of severe hypoglycemia have identified a specific structural lesion in the hippocampus in individuals with insulin-treated diabetes (43,44).

The results of the present study have clearly demonstrated the short-term detrimental effects of acute hypoglycemia on memory and are consistent with these observations. However, the present study also demonstrated that other domains of cognition are impaired during moderately severe hypoglycemia, and there is now considerable evidence that acute neuroglycopenia causes rapid deterioration in psychomotor performance across a wide range of cognitive domains (3). It is feasible, therefore, that the impairment of memory performance observed in this study reflects a transient global brain dysfunction induced by hypoglycemia rather than specific vulnerability of memory processes to hypoglycemia. The very large effect size associated with the Validation Span Test, in particular, may be a consequence of task complexity. However, performance in another test of working memory was also very significantly impaired, to chance levels, in the same diabetic and nondiabetic subjects (45).

Individuals with insulin-treated diabetes are frequently exposed to varying degrees of hypoglycemia, and current therapeutic policies that strive to achieve strict glycemic control may promote more frequent hypoglycemic events. The re-

sults of the present study demonstrate that many individuals with type 1 diabetes are subject to substantial impairments of memory function during hypoglycemia in their everyday lives, which may have important practical implications for daily activities, including effective working ability and driving performance (46).

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Acute Hyperglycemia Alters Mood State and Impairs Cognitive Performance in People With Type 2 Diabetes

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OBJECTIVE — To examine the effects of acute hyperglycemia on cognitive function and mood in people with type 2 diabetes.

RESEARCH DESIGN AND METHODS — Twenty subjects with type 2 diabetes, median age 61.5 years (range 53.1–72.0), known duration of diabetes 5.9 years (range 2.8–11.2), BMI 29.8 kg/m² (range 22.0–34.6), and HbA_{1c} 7.5% (range 6.7–8.4) were studied. Treatment modalities varied from antidiabetic medications to insulin. A hyperinsulinemic glucose clamp was used to maintain arterialized blood glucose at either 4.5 (euglycemia) or 16.5 mmol/l (hyperglycemia) on two occasions in a randomized and counterbalanced fashion. Tests of information processing, immediate and delayed memory, working memory, and attention were administered, along with a mood questionnaire, during each experimental condition.

RESULTS — Speed of information processing, working memory, and some aspects of attention were impaired during acute hyperglycemia. Subjects were significantly more dysphoric during hyperglycemia, with reduced energetic arousal and increased sadness and anxiety.

CONCLUSIONS — During acute hyperglycemia, cognitive function was impaired and mood state deteriorated in a group of people with type 2 diabetes. These findings are of practical importance because intermittent or chronic hyperglycemia is common in people with type 2 diabetes and may interfere with many daily activities through adverse effects on cognitive function and mood.

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Diabetes is associated with rapid fluctuations in blood glucose. Hyperglycemia is a frequent consequence of the relative or absolute insulin deficiency that is intrinsic to diabetes, and hypoglycemia is a common side effect of treatment with insulin and some antidiabetic medications (1). Because the brain is dependent on a continuous supply of glucose as its principal source of energy, changes in blood glucose concentration rapidly affect cerebral function. The adverse effects of acute hypoglycemia on cognitive function and on mood are rec-

ognized (2,3). However, less is known about the effects of acute hyperglycemia on cerebral function. Anecdotal descriptions by patients with diabetes suggest that when blood glucose is elevated, changes in mood (such as increased irritability and feelings of diminished well-being) occur and rapid thinking is more difficult.

Published data on the effects of acute hyperglycemia on cognitive function are contradictory. Two studies (4,5) have demonstrated impaired language skills and reduced IQ during hyperglycemia

compared with euglycemia. Other studies have shown no effect of acute hyperglycemia on cognitive function (6–8) or mood (9). However, in the study by Gschwend et al. (6), only two tests were used to assess cognitive function, and in the studies by Draelos et al. (8) and Weinger et al. (9) the study cohorts had chronically poor metabolic control, which may have allowed cerebral adaptation to occur in response to prevailing high blood glucose concentrations. A further study by Sindrup et al. (10) showed that short-term hyperglycemia (blood glucose concentration 17.1 mmol/l) with physiological hyperinsulinemia was associated with increased sensory nerve conduction velocity and decreased motor latency in nondiabetic subjects.

Earlier studies have been confined to people with type 1 diabetes. Evidence is accumulating that people with type 2 diabetes are at risk of developing cognitive impairment (11,12). This is probably a consequence of synergistic interaction between metabolic derangements associated with diabetes and the structural and functional changes that occur within the central nervous system as part of the normal aging process. People with type 2 diabetes may be susceptible to cognitive dysfunction during short-term changes in blood glucose concentration. The present study examined the effects of acute hyperglycemia on a range of important cognitive function and key mood states in a group of people with type 2 diabetes.

RESEARCH DESIGN AND METHODS

Twenty adults (12 men) with type 2 diabetes were studied, following recruitment from the diabetes outpatient clinic at the Royal Infirmary of Edinburgh. Baseline characteristics included median age 61.5 years (range 53.1–72.0), BMI 29.8 kg/m² (range 22.0–34.6), known duration of diabetes of 5.9 years (range 2.8–11.2), and HbA_{1c} 7.5% (range 6.7–8.4). HbA_{1c}, recorded in the month before the study, was measured by high-performance liquid chromatography (Variant II Hemoglobin

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A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

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Testing System; BioRad Diagnostics Group, Hercules, CA) with a local nondiabetic reference range of 4.3–6.5%. Three of the subjects required insulin to treat their diabetes, five were taking a single antidiabetic drug, nine were taking a combination of antidiabetic drugs, and three were taking an antidiabetic drug and once-daily isophane (NPH) insulin. None of the participants had a history of any other chronic disease, previous head injury, seizure, blackouts, alcohol or drug abuse, or psychiatric illness. Subjects were screened for diabetes complications and were excluded if they had evidence of microvascular disease, with the exception of background retinopathy. The presence of retinopathy was ascertained using direct ophthalmoscopy, peripheral neuropathy by clinical examination, and nephropathy was presumed by the presence of established microalbuminuria. Ethical permission for the study was approved by the local medical research ethics committee. All subjects gave written informed consent for participation in the study.

Each subject participated in two laboratory sessions that were separated by at least 2 weeks. A modified hyperinsulinemic glucose clamp (13) was used to maintain blood glucose at a predetermined level. In each study condition, the arterialized blood glucose concentration was initially stabilized at 4.5 mmol/l for a period of 30 min. In the euglycemia condition, the blood glucose concentration was thereafter maintained at 4.5 mmol/l throughout the study. In the hyperglycemia condition, blood glucose was raised to 16.5 mmol/l over a period of 20 min. The blood glucose concentration was maintained at the predetermined target level for a further 10 min before commencing cognitive testing and was kept at this level for a further 80 min while the tests were administered. The subjects were not informed which arm of the study was being undertaken on each occasion, and the two sessions were performed in a randomized and counterbalanced fashion.

Cognitive function tests

Validated tests of information processing, tests of memory, and tests of attention were administered during each study condition.

Test of information processing

Trail Making B. This test, which was run on a handheld computer (14,15), assesses

complex visual scanning and has a motor component.

Digit Symbol test. This is a test of coding performed at speed (16).

Reaction Time test. This is a test of psychomotor speed and information processing (17). SDs of the Simple and Four-Choice Reaction Times were also calculated, providing a measure of intra-individual variability, and the coefficient of variation was calculated.

Tests of memory. The memory and learning tests that were used in this study were chosen because they have previously been shown (18) to be sensitive to metabolic disturbances such as hypoglycemia. **Verbal memory tests.** 1) Auditory Verbal Learning Test, immediate and delayed. This is a test of immediate memory capacity, retrieval efficiency, and learning. The delayed component measures longer-term retention (19). 2) Logical Memory Test, immediate and delayed. The Logical Memory test (20), a test of verbal learning, measures immediate and delayed recall following auditory presentation.

Visual memory tests. 1) Visual Reproduction, immediate and delayed. This test measures immediate and delayed recall following nonverbal visual presentation (20). 2) Benton Visual Retention Test. This is a test of immediate visual recall (21).

Working memory tests. 1) Digit Span Forwards and Backwards. In this test, a series of lists of numbers is presented verbally to the subject, and the lists progressively increase in length (20). 2) Letter/Number Sequencing. A series of lists of numbers mixed with letters is presented verbally (20).

Tests of attention. The Test of Everyday Attention battery (22) was used to measure attention and includes tests of visual selective, auditory selective, divided, and sustained attention and of attention switching.

Parallel versions of the tests are available for the Auditory Verbal Learning Test, Logical Memory, the Benton Visual Retention Test, and the Test of Everyday Attention battery, and in the present study these were used to minimize a learning effect between the two study conditions. Throughout the study, the battery of tests was carried out in a fixed order.

Mood questionnaire

The University of Wales Institute of Science and Technology (UWIST) mood adjective checklist was used to document changes in mood experienced by the sub-

jects during the two studies (23). There are three main mood states: energetic arousal (feeling lively/active versus tired/sluggish), tense arousal (feeling anxious/nervous versus relaxed/calm), and hedonic tone (feeling happy versus sad).

Statistical analysis

The results were analyzed independently for each test. A general linear model (repeated-measures ANOVA) was used with order of session (euglycemia-hyperglycemia or hyperglycemia-euglycemia) as a fixed effect, and condition (euglycemia or hyperglycemia) as a within-subjects factor. The above models were repeated with the following variables added singly and separately: sex as a fixed effect and with age, glucose infusion rate, HbA_{1c}, and the three mood states as covariates. The order effect was retained in the models when reported in the results. A *P* value <0.05 was considered to be significant. The *P* values reported in the tables for the core model (including only condition and order) were generated from analyses without including sex and the covariates. The *P* value reported in the tables for the additional fixed effect of sex and the covariates refers to analyses in which these were added singly to the core model. Effect size was calculated using η^2 . An η^2 score of 0.25–0.5 indicates a moderate effect size. Power was calculated using nQuery, which gives δ^2 -based power for univariate repeated-measures ANOVA. α was set at 0.05 (*n* = 20). Between-level correlation was set at zero, which offers a conservative power estimate because correlations are generally modest and positive. The power to detect an effect size of 0.25 = 85% and to detect an effect size of 0.50 = 98%. All analyses were performed using SPSS version 11.0 for Windows.

RESULTS—A stable blood glucose plateau was achieved during each study condition. The mean (\pm SD) arterialized blood glucose concentration during the euglycemia condition was 4.5 ± 0.2 mmol/l and during the hyperglycemia condition was 16.7 ± 0.6 mmol/l. Statistical analysis revealed that no significant order effects had occurred for any of the outcome variables of this study. The significant effects of acute hyperglycemia on cognitive function remained significant in all analyses after controlling for sex, age, HbA_{1c}, and glucose infusion rate.

Table 1—Results of tests of information processing during euglycemia and hyperglycemia in 20 adults with type 2 diabetes

Subtest	Euglycemia	Hyperglycemia	P	F	Eta ²	Age	Sex	GIR	HbA _{1c}	TA	EA	HT
Digit Symbol Substitution test	70.2 ± 8.4	66.8 ± 7.8	0.03	5.64	0.24	0.38	0.26	0.65	0.71	0.64	0.57	0.96
Trail Making B test	40.9 ± 7.8	43.9 ± 8.0	0.04	4.77	0.23	0.74	0.77	0.34	0.32	0.38	0.39	0.39
Simple Reaction Time test												
Mean	359.9 ± 84.6	373.7 ± 72.3	0.10	2.99	0.14	0.81	0.43	0.76	0.33	0.41	0.28	0.08
SD	73.3 ± 11.2	77.5 ± 17.6	0.10	0.47	0.15	0.96	0.18	0.98	0.81	0.50	0.45	0.68
Coefficient of variation	21.09 ± 6.86	21.82 ± 6.01	0.38	0.82	0.01	0.92	0.19	0.82	0.65	0.31	0.09	0.16
Four-Choice Reaction Time test												
Mean	710.0 ± 116.7	775.7 ± 122.9	<0.0001	23.05	0.56	0.55	0.12	0.48	0.92	0.46	0.76	0.74
SD	133.1 ± 23.6	139.5 ± 41.2	0.09	0.68	0.18	0.09	0.48	0.60	0.70	0.67	0.34	0.26
Coefficient of variation	18.73 ± 2.77	18.22 ± 4.02	0.64	0.23	0.01	0.06	0.76	0.61	0.68	0.80	0.41	0.95
Number of errors	0.5 ± 0.1	0.4 ± 0.1	0.43	0.54	0.09	0.54	0.65	0.36	0.62	0.51	0.22	0.31

Data are means ± SD. The effects of age, sex, glucose infusion rate (GIR), HbA_{1c}, and mood as covariates are also shown (all *P* values). EA, energetic arousal; HT, hedonic tone; TA, tense arousal.

Tests of information processing

The results of these tests are summarized in Table 1. During acute hyperglycemia, performance was significantly impaired in the Trail Making B, Digit Symbol, and Four-Choice Reaction Time tests. Performance in the Simple Reaction Time test was not significantly impaired during hyperglycemia. The coefficient of variation for the Simple and Four-Choice Reaction Time tests was not significantly different between the euglycemia and hyperglycemia conditions.

Tests of memory

The results of the memory function tests are summarized in Table 2. Acute hyperglycemia had no significant effect on tests of immediate or delayed memory. Performance in two tests of working memory (Digit Span Backwards, Letter/Number

Sequencing) was impaired during acute hyperglycemia.

Tests of attention

The results of the tests of attention are summarized in Table 3.

Visual selective attention. The mean number (±SD) of map symbols circled in 1 min was significantly fewer during hyperglycemia. The number of symbols circled in 2 min was also lower during hyperglycemia, but the difference did not achieve statistical significance. In the telephone search task, no difference was demonstrated between euglycemia and hyperglycemia in the number of symbols located. The mean (±SD) time taken to complete the task during euglycemia was significantly faster than that during hyperglycemia.

Auditory selective attention. The auditory elevator test with reversal was significantly affected by hyperglycemia. However, performance in the elevator with distraction test was not impaired.

Sustained attention. Sustained attention was not affected by hyperglycemia.

Attention switching. In the visual elevator task, no difference was observed in the raw score between the two study conditions. However, a significantly longer time was required to complete each switch of the visual elevator task during hyperglycemia.

Divided attention. In the task that involved search of a telephone directory while counting, no significant difference was observed in the number of symbols that were located during either study condition. The time taken to complete the task was longer during hyperglycemia com-

Table 2—Results of tests of memory during euglycemia and hyperglycemia in 20 adults with type 2 diabetes

Subtest	Euglycemia	Hyperglycemia	P	F	Eta ²	Age	Sex	GIR	HbA _{1c}	TA	EA	HT
Immediate memory												
Immediate Logical Memory	27.2 ± 4.3	26.1 ± 4.1	0.16	2.13	0.11	0.93	0.16	0.67	0.41	0.52	0.47	0.88
Immediate AVLT	35.0 ± 5.7	32.4 ± 4.9	0.09	3.33	0.16	0.78	0.12	0.43	0.56	0.46	0.46	0.38
Benton Visual Retention Test	6.2 ± 1.2	5.9 ± 0.9	0.30	1.16	0.06	0.82	0.88	0.31	0.32	0.21	0.63	0.18
Visual Reproduction	76.9 ± 7.6	77.9 ± 7.5	0.51	0.44	0.02	0.64	0.95	0.79	0.39	0.30	0.12	0.66
Working memory												
Digit Span Forwards	9.6 ± 1.3	9.2 ± 1.6	0.33	0.99	0.05	0.53	0.18	0.53	0.41	0.48	0.54	0.61
Digit Span Backwards	8.6 ± 1.5	7.9 ± 1.3	0.01	5.01	0.30	0.13	0.09	0.49	0.36	0.61	0.55	0.89
Letter/Number Sequencing	10.4 ± 1.9	9.4 ± 1.6	0.02	6.94	0.25	0.24	0.10	0.60	0.38	0.17	0.31	0.87
Delayed memory												
Delayed Logical Memory	13.2 ± 2.1	12.3 ± 2.2	0.09	3.16	0.15	0.54	0.18	0.78	0.51	0.85	0.68	0.72
Delayed AVLT	8.2 ± 2.2	7.8 ± 1.9	0.26	6.23	0.07	0.25	0.46	0.94	0.26	0.62	0.40	0.06
Delayed Visual Reproduction	14.1 ± 9.2	10.8 ± 8.2	0.08	3.58	0.17	0.41	0.31	0.36	0.76	0.59	0.31	0.70

Data are means ± SD. The effects of age, sex, glucose infusion rate (GIR), HbA_{1c}, and mood as covariates are also shown (all *P* values). AVLT, Auditory Verbal Learning Test; EA, energetic arousal; HT, hedonic tone; TA, tense arousal.

Table 3—Results of tests of attention during euglycemia and hyperglycemia in 20 adults with type 2 diabetes

Subtest	Euglycemia	Hyperglycemia	P	F	Eta ²	Age	Sex	GIR	HbA _{1c}	TA	EA	HT
Visual selective attention												
Map Search (1 min)	35.0 ± 6.7	32.2 ± 5.1	0.04	3.36	0.22	0.17	0.21	0.39	0.44	0.31	0.26	0.41
Map Search (2 min)	64.8 ± 7.4	62.5 ± 8.7	0.31	1.07	0.06	0.60	0.57	0.57	0.41	0.27	0.44	0.72
Telephone Search (raw score)	3.6 ± 0.7	3.7 ± 0.6	0.34	0.95	0.05	0.11	0.19	0.30	0.62	0.44	0.51	0.96
Telephone Search (total time)	58.7 ± 8.3	67.0 ± 13.1	0.04	2.91	0.27	0.43	0.24	0.56	0.71	0.61	0.16	0.76
Auditory selective attention												
Elevator Counting (with distraction)	8.8 ± 1.1	8.3 ± 1.3	0.12	2.71	0.13	0.56	0.50	0.83	0.47	0.32	0.28	0.06
Elevator Counting (with reversal)	5.3 ± 1.7	4.4 ± 1.4	0.01	7.44	0.29	0.12	0.08	0.49	0.50	0.38	0.24	0.17
Attention switching												
Visual Elevator (raw score)	8.9 ± 1.1	8.5 ± 0.9	0.17	2.01	0.10	0.69	0.85	0.08	0.90	0.19	0.87	0.59
Visual Elevator (switch time)	4.3 ± 1.0	4.7 ± 0.9	0.001	17.44	0.49	0.15	0.43	0.60	0.26	0.09	0.63	0.20
Divided attention												
Telephone Search (with counting total time)	62.3 ± 9.5	67.2 ± 17.1	0.10	2.71	0.12	0.56	0.25	0.78	0.53	0.42	0.21	0.60
Telephone Search (with counting time per target)	1.0 ± 0.6	1.2 ± 0.6	0.05	4.45	0.20	0.31	0.37	0.59	0.62	0.46	0.54	0.68
Sustained attention												
Elevator Counting	6.6 ± 0.68	6.7 ± 0.67	0.79	0.07	0.01	0.1	0.18	0.68	0.38	0.30	0.31	0.27
Lottery	9.6 ± 0.7	9.7 ± 0.6	0.63	0.24	0.01	0.48	0.18	0.93	0.67	0.27	0.82	0.26

Data are means ±SD. The effects of age, sex, glucose infusion rate (GIR), HbA_{1c}, and mood as covariates are also shown (all P values). EA, energetic arousal; HT, hedonic tone; TA, tense arousal.

pared with during euglycemia, but this difference was not significant. The timer-per-target score, which is the ratio of the number of circled symbols divided by the time taken for the task, was significantly higher during acute hyperglycemia.

Mood

The results of the mood questionnaire are shown in Fig. 1. Hedonic Tone and Energetic Arousal scores were significantly lower during hyperglycemia (decreased happiness and alertness), whereas feelings of Tense Arousal were greater (increased agitation).

CONCLUSIONS — The present study demonstrated that acute hyperglycemia in people with type 2 diabetes significantly impaired speed of information processing, working memory, and some aspects of attention. It also had a profound detrimental effect on key mood states.

It was apparent from the results of the present study that performance was impaired during acute hyperglycemia in tests that required a speedy response, suggesting that accuracy was preserved at the expense of speed. For example, in the Map Search, a test of visual selective attention, significantly fewer symbols were identified after 1 min during hyperglycemia when compared with euglycemia.

lycemia. However, in the overall 2-min score no significant difference was observed, which indicates that a “ceiling effect” is reached after 2 min. In the Telephone Search test and the Telephone Search While Counting test, no significant difference could be discerned in the total number of symbols located during either study condition, but

the time taken to complete these tests was significantly longer during hyperglycemia. In the Visual Elevator task no significant difference in the raw score was observed during hyperglycemia compared with euglycemia, but the time taken to complete each switch was significantly greater during hyperglycemia.

The cognitive domains that were

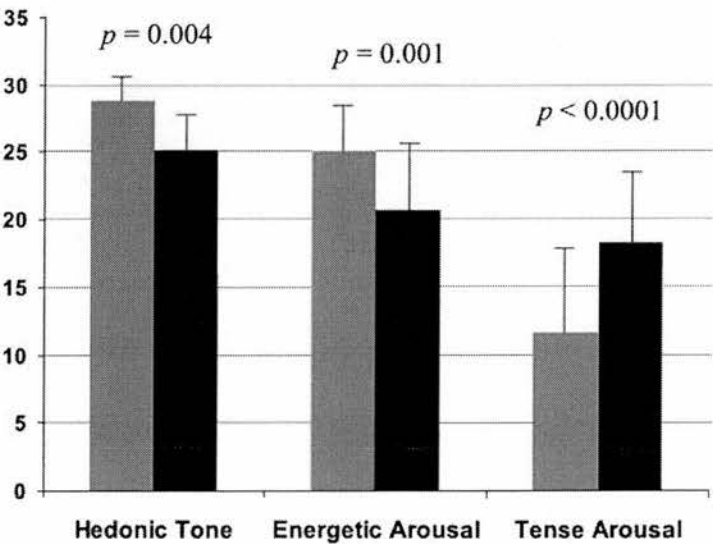


Figure 1—Graph showing mean (±SD) scores of the UWIST mood adjective checklist during euglycemia (□) and hyperglycemia (■).

most adversely affected by hyperglycemia in people with type 2 diabetes were information processing speed and working memory. Performance in three of the four processing speed tests was significantly impaired during hyperglycemia. In the memory domain, the only two tests affected were of working memory; acute hyperglycemia did not have a significant detrimental effect on tests of immediate and delayed memory. This suggestion is supported by the "attention" results, which also demonstrated a reduction in speed of information processing (rather than accuracy), and this was particularly apparent in tests of attention that made demands on working memory, e.g., visual elevator switch time, which requires storage and manipulation of information.

In addition to the specific cognitive demands of the tasks affected by hyperglycemia, impairment of performance in these tests may reflect their relative complexity. Davis et al. (5) demonstrated that in children with type 1 diabetes cognitive function was impaired during acute hyperglycemia but that this was limited to complex tests of cognitive ability. In the present study, performance in relatively simple tests of information processing (Simple Reaction Time), memory (tests of immediate and delayed verbal and visual memory, Digit Span Forwards), and attention (Lottery and Elevator Counting) was not affected by acute hyperglycemia. However, during acute hyperglycemia a significant decrement in performance was observed in comparatively more difficult tasks, such as the Four-Choice Reaction Time test, Digit Span Backwards, Letter/Number Sequencing, and Elevator Counting with Reversal.

Mood was also adversely affected by acute hyperglycemia. The changes in mood included increased feelings of agitation and anxiety (increased tense arousal), increased feelings of tiredness and lethargy (decreased energetic arousal), and decreased feelings of happiness (decreased hedonic tone). Further analysis of the cognitive function test results with mood as a covariate demonstrated that the impairment in cognitive function occurred independently of changes in mood state.

The results of the present study indicate that acute hyperglycemia has a significantly adverse effect on various aspects of cognitive function and mood. However, with the exception of the Four-Choice Re-

action Time test, the η^2 values for the tests of cognitive function were modest. The study had 80% power to detect an effect size of $\delta^2 = 0.21$. Therefore, we were not able to detect small effect sizes. However, at that level, they might not be of great practical significance. The number of subjects included in the study is large by comparison with most glucose clamp studies. Apart from giving more confidence in the results, this was also done because of the relatively large numbers of cognitive outcomes, which were included because they have been found to be sensitive to the effects of hypoglycemia. These obviously carry the possibility of type I errors, and it will be useful to carry out a further study using a larger sample to try and replicate these findings. Nevertheless, it was important for this first, systematic study of the cognitive effects of hyperglycemia to include the main domains of cognitive function. The effects of acute hypoglycemia on cognitive function have been observed to return to normal within 90 min of the restoration of euglycemia (24,25). It is not known whether the effect of hyperglycemia on cognitive function or mood is sustained or protracted, and this will require further investigation.

Some indirect evidence suggests that hyperglycemia may have adverse effects on cerebral function. For example, acute hyperglycemia has been shown (26) to enhance cerebral damage resulting from ischemic stroke. There is also accumulating evidence to suggest that chronic hyperglycemia, as indicated by surrogate markers such as the presence of peripheral neuropathy or retinopathy, is involved in the pathogenesis of the cognitive impairment associated with diabetes (27–29).

The findings of the present study are in concordance with anecdotal reports from people with type 2 diabetes and are supported by the results of two recent studies. Cox et al. (30) have demonstrated changes in mood and impairment in cognitive function during acute hyperglycemia in people with type 1 and type 2 diabetes, and a study (31) in Canada has shown impairment of cognitive function in people with type 2 diabetes following oral ingestion of 50 g of carbohydrate. Taken together, these findings are of practical importance to people with type 2 diabetes in whom exposure to moderate and intermittent hyperglycemia is common. Working memory and processing

speed are fundamental aspects of cognition in everyday life. The deleterious effects of hyperglycemia on cognitive function and mood states may significantly interfere with many activities of daily living and may influence therapeutic strategies aimed at treating postprandial hyperglycemia.

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Vessel wall stiffness in type 1 diabetes and the central hemodynamic effects of acute hypoglycemia

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Sommerfield AJ, Wilkinson IB, Webb DJ, Frier BM. Vessel wall stiffness in type 1 diabetes and the central hemodynamic effects of acute hypoglycemia. *Am J Physiol Endocrinol Metab* 293: E000–E000, 2007. First published August 28, 2007; doi:10.1152/ajpendo.00114.2007.—Objective: To examine the effects of intravenous insulin and acute hypoglycemia on arterial wall stiffness and central hemodynamic responses in adults with and without type 1 diabetes. Research Design and Methods: In 30 young male volunteers [10 nondiabetic (*Group 1*); 10 with type 1 diabetes, <5 yr duration (*Group 2*); 10 with type 1 diabetes, >15 yr duration (*Group 3*)], intravenous insulin was administered to provoke an acute autonomic reaction (R) to hypoglycemia. Heart rate, peripheral blood pressure, and pulse wave analysis (radial artery) were monitored. Augmentation index (AIx), a measure of arterial wall stiffness and wave reflection, and central arterial pressure were recorded. Results: At baseline, no significant differences were observed between *Groups 1* and *2* in either AIx or in central arterial pressure, but in *Group 3*, both measures were significantly higher. All three groups exhibited similar responses to intravenous infusion of insulin and to hypoglycemia: AIx fell progressively from baseline to R, peripheral systolic blood pressure increased, and central systolic pressure decreased. Conclusion: Compared with age- and sex-matched nondiabetic controls, people who had type 1 diabetes for a long duration had increased stiffness of vessel walls. The opposing responses in peripheral and central systolic pressures during hypoglycemia may be related to the reduction in AIx, which causes diminished amplification of the systolic pressure wave. Changes in AIx are probably mediated by a direct action of insulin on arterial endothelium, or changes in heart rate. These functional changes may contribute to the increased cardiovascular morbidity that is associated with type 1 diabetes of long duration.

arterial wall stiffness; central arterial pressure; insulin

PEOPLE WITH TYPE 1 DIABETES are frequently exposed to acute hypoglycemia, which, in humans, is associated with profound hemodynamic changes, including an increase in heart rate and stroke volume, increased myocardial contractility, and a rise in cardiac output (14, 15). Changes in peripheral blood pressure include an increase in systolic and a decrease in diastolic pressure, with no change in mean arterial pressure. The effects on central arterial pressure are not known. Acute hypoglycemia also has significant, albeit transient, effects on peripheral blood that encourage hemostasis and increase blood viscosity (15). These changes influence capillary blood flow and in some tissues may predispose to intravascular stasis during hypoglycemia. The hemodynamic and hematological changes that occur during acute hypoglycemia do not appear to compromise

vascular perfusion in the normal healthy vasculature, but they may have potentially adverse effects in people with diabetes, in whom endothelial dysfunction and vascular disease are common, and in whom premature atherosclerosis and cardiovascular disease are often present. Indeed, acute hypoglycemia may precipitate an acute vascular event or a dysrhythmia in people who have preexisting coronary heart disease (15). Angina and acute myocardial infarction have been documented, albeit infrequently and anecdotally, as the direct consequences of acute hypoglycemia (35).

Arterial blood pressure is usually recorded noninvasively at the brachial artery by sphygmomanometry. It is assumed that the pressure is the same throughout the arterial tree, and that it represents an accurate index of aortic peak pressure and of left ventricular systolic pressure, and so of left ventricular afterload. However, blood pressure measured in the peripheral circulation is not an accurate estimate of central pressure because of amplification of the pressure pulse between central and peripheral arteries (34, 43). Normally, there is amplification of the pulse pressure between the aorta and brachial artery (24). It is the aortic, rather than the brachial, pressure that determines left ventricular workload (42, 47).

Central arterial pressure and arterial wall stiffness, which are independent predictors of cardiovascular morbidity (1, 3, 20, 25, 31), can be studied noninvasively using pulse wave analysis (30, 32, 44, 51), which requires the external application of a micromanometer-tipped probe over a peripheral artery (6, 8). Pulse wave analysis allows the measurement of central arterial pressure, and the degree of its augmentation by pulse wave reflection, to be studied reliably and noninvasively (32). The system utilizes applanation tonometry, with the external application of a micromanometer-tipped probe to record peripheral pulse waveforms. The radial artery is usually used for these measurements because of its close proximity to and the support provided by nearby bony structures (6, 8). Changes occur in the pressure contour as it travels from the aorta to more peripheral sites, so that pressures at the radial artery cannot be used directly as a surrogate to predict central pressure in the aorta. However, it is possible to estimate the central aortic pressure wave from measurements of radial artery tonometry with the use of a mathematical transformation (8). Several studies have validated the accuracy and reliability of pulse wave analysis (44, 51). Contraction of the left ventricle generates a pressure wave that travels along the major arteries until it meets sites of resistance (high-resistance arterioles), and then the wave is reflected back to the heart (29). The stiffness of the arterial wall

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influences the velocity of the reflected wave. In healthy young people, the reflected wave reaches the heart during diastole and, by increasing diastolic pressure, enhances coronary perfusion. However, when arterial stiffening has developed, the increased pulse wave velocity of the stiffened arteries leads to earlier reflection of the wave, so that it returns to the heart during late systole (31, 33). The increase in systolic pressure that results from this earlier reflection of the pressure wave is known as "augmentation," with augmentation index (AIx) measured as the augmented pressure expressed as a percentage of the pulse pressure. AIx increases with age and in disease states such as hypertension (28) and hypercholesterolemia (49). It is also influenced by heart rate; a decrease in AIx is observed with an increase in heart rate (45). Central arterial pressure and waveform convey important information about cardiovascular status (21, 27, 31).

In view of the frequency of exposure to acute hypoglycemia in people with diabetes and the potential effects of acute hypoglycemia on the cardiovascular system, it is valuable to establish the effects of acute hypoglycemia on central arterial pressure and left ventricular load. The present study sought to measure arterial wall stiffness and the central hemodynamic responses to the administration of intravenous insulin and the induction of acute hypoglycemia in people with type 1 diabetes of varying duration and in nondiabetic subjects.

METHODS

Subjects. To avoid potential gender differences in the counterregulatory hormonal responses to hypoglycemia (10), three groups of male subjects, matched for age, body mass index (BMI), and peripheral blood pressure, were studied: 10 nondiabetic volunteers (*Group 1*), 10 subjects with type 1 diabetes of short duration (<5 yr) (*Group 2*), and 10 subjects with type 1 diabetes of longer duration (>15 yr) (*Group 3*). Subjects with diabetes were matched for glycated hemoglobin (HbA1c) and were recruited from the outpatient clinic at the Department of Diabetes at the Royal Infirmary of Edinburgh. All patients were receiving treatment with a basal-bolus insulin regimen consisting of a preprandial short-acting insulin analog and a long-acting insulin analog, administered once daily. Blood pressure and plasma cholesterol concentration were measured before inclusion in the study. HbA1c was measured by HPLC (Variant II Hemoglobin Testing System; Bio-Rad Diagnostics Group, Hercules, CA), with a local Diabetes Control and Complications Trial-adjusted nondiabetic reference range of 4.3–6.5%. Subjects were excluded from the study if there was a history of hypertension (blood pressure $>140/80$ mmHg), hypercholesterolemia (total cholesterol >5.0 mmol/l), or other chronic disease or a history of a previous head injury, alcohol or drug abuse, seizures, or blackouts. All of the subjects were nonsmokers, had no intercurrent illness, and were not taking any regular medication (other than insulin for diabetes). Subjects with diabetes were excluded if they had any evidence of microvascular disease other than background diabetic retinopathy (2 subjects in *Group 2* and 5 subjects in *Group 3*). The presence of retinopathy was determined by direct ophthalmoscopy, peripheral neuropathy by clinical examination, and nephropathy by the presence of microalbuminuria (albumin-to-creatinine ratio >2.5 mg/mmol for women and >3.5 mg/mmol for men). All potential participants underwent standard autonomic function testing (13) before inclusion in the study, and any with autonomic dysfunction were excluded. Subjects were also excluded if they had a history of impaired awareness of hypoglycemia.

Studies were postponed if a subject had experienced hypoglycemia in the preceding 48 h, and subjects were required to measure their blood glucose frequently during this period, including bedtime tests on the evening before the study to identify biochemical hypoglycemia.

Ethical permission for the study was given by the Lothian Research Ethics Committee. All subjects gave their written, informed consent.

Procedure. Each study session began at 0800 after a 10- to 12-h overnight fast. The subjects with type 1 diabetes omitted their insulin on the morning of the study. A hyperinsulinemic glucose clamp (12) was used initially to maintain euglycemia (blood glucose concentration 4.0–7.0 mmol/l) for 20 min before the induction of acute hypoglycemia. Because of the variable fasting blood glucose level in people with diabetes, it took a variable time to achieve euglycemia (usually between 20 and 30 min). An intravenous cannula was inserted retrogradely into a vein on the dorsum of the nondominant hand for regular blood sampling. The hand was placed in a heated blanket to arterialize the venous blood. A second intravenous cannula was inserted into a vein in the antecubital fossa of the same arm for infusion of human soluble insulin (Humulin S; Eli Lilly) and 20% dextrose. Intradermal lignocaine (1%) was used for insertion of each cannula. Insulin was infused at a constant rate of $60 \text{ mU} \cdot \text{m}^{-2} \cdot \text{min}^{-1}$ using an IMED Gemini PCI pump (Alaris Medical Systems, San Diego, CA). A variable intravenous infusion of 20% dextrose was given simultaneously. The rate of dextrose infusion was varied according to the arterialized blood glucose concentration. This was measured at the bedside using the glucose oxidase method (Yellow Springs Instrument 2300 Stat, Yellow Springs, OH). Blood glucose concentration was measured every 3 min initially and then every 5 min once a stable level had been achieved.

Because the purpose of inducing acute hypoglycemia was to provoke an acute autonomic reaction with profound activation of the sympathoadrenal system, a rapid fall in blood glucose was then induced by infusing insulin intravenously at a rate of $2 \text{ mU} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$. This caused a controlled decline in blood glucose to a level that triggered the autonomic reaction associated with hypoglycemia. This method of inducing hypoglycemia simulates the development of hypoglycemia under everyday conditions and cannot be induced using the hyperinsulinemic glucose clamp technique. Any variability among subjects in the rate of fall in blood glucose and the depth of the hypoglycemia achieved is inconsequential, as the intention is to lower blood glucose to the blood glucose threshold at which autonomic activation is stimulated.

The autonomic reaction (designated as R) was identified by an abrupt rise in heart rate (15% above baseline), the rapid onset of autonomic symptoms, such as sweating and tremor, and a rapid rise in peripheral systolic blood pressure. When the R occurred, the clock timer was reset to zero, and all subsequent measurements were timed relative to this point. This accommodates the variable time interval that occurs among individuals in the development of the R using the insulin infusion technique (9). As soon as R had occurred, the insulin infusion was discontinued, and hypoglycemia was reversed using an intravenous infusion of 20% dextrose to restore normoglycemia. All subjects consumed a meal on completion of the study.

Measurements. During the study, arterialized blood glucose was measured at the bedside (Yellow Springs Instrument 2300 Stat) every 5 min until R and every 15 min thereafter until R+30 min. The maximal hemodynamic changes in response to hypoglycemia occur in the 30 min following the onset of the R (14). Pulse wave analysis at the radial artery was therefore performed every 5 min until R and every 5 min thereafter until R+30 min. Heart rate was monitored continuously using a pulse oximetry probe. Peripheral blood pressure was recorded every 5 min using a digital automated sphygmomanometer.

The Edinburgh Hypoglycemia Scale (11), a validated subjective self-rating questionnaire, was used to document the symptoms of hypoglycemia experienced by the subjects during the study. The symptoms of hypoglycemia were classified as autonomic (hunger, palpitations, sweating, shaking), neuroglycopenic (drowsiness, confusion, inability to concentrate, speech difficulty, blurred vision), and nonspecific (malaise, nausea, headache). Each symptom was graded on a Likert scale of 1 to 7 (1 = not present, 7 = very intense). The

questionnaire was applied at baseline and at 5-min intervals thereafter until the onset of the R. It was repeated at R+30 min.

Statistics. A general linear model (repeated-measures ANOVA) was used to compare means within individual groups. Between groups, an ANOVA with post hoc *t*-tests was used. Correlation analyses for changes in AIx and insulin dose and duration of the study were performed using the Pearson correlation. A *P* value <0.05 was considered to be significant. All analyses were performed using SPSS version 11.0 for Windows. All results are expressed as means \pm SD.

RESULTS

The clinical characteristics of the subjects in the study are shown in Table 1.

Changes in blood glucose concentration. The blood glucose concentrations (means \pm SD) at baseline were 4.3 ± 0.5 mmol/l in Group 1 (nondiabetic) and 8.4 ± 2.1 and 8.9 ± 3.1 mmol/l in Groups 2 and 3 (diabetic), respectively. Following application of the euglycemic clamp, the blood glucose concentrations were stabilized at 4.2 ± 0.1 mmol/l (Group 1), 4.3 ± 0.2 mmol/l (Group 2), and 4.3 ± 0.2 mmol/l (Group 3). The nadir blood glucose concentrations at R were 2.9 ± 0.3 mmol/l (Group 1), 2.8 ± 0.3 mmol/l (Group 2), and 2.6 ± 0.3 mmol/l (Group 3) (*P* = 0.2).

Peripheral blood pressure. No significant differences were observed in the peripheral systolic, diastolic, and mean arterial pressures among the three groups at baseline. In all three groups of subjects, an increase in peripheral systolic blood pressure and a decrease in peripheral diastolic pressure occurred at R compared with baseline (Table 2). Systolic and diastolic pressures had returned to baseline levels by R+30 min in all three groups (Table 2).

Central arterial pressure. Central systolic blood pressure at baseline was significantly higher in Group 3 compared with Groups 1 and 2 (Table 1). A small, but significant, reduction in central systolic pressure was observed at R in all three groups compared with the respective baseline levels (Table 2). No change in central diastolic blood pressure was observed throughout the study in any of the three groups (Table 2).

Heart rate and autonomic symptom score. The induction of hypoglycemia and the onset of the R were associated with significant increments of heart rate and autonomic symptom score in all three groups compared with baseline levels (Table

2). In all groups, the heart rate and the autonomic symptom score had returned to baseline values at R+30 min.

AIx. At baseline, no difference in AIx was discernible between Group 1 and Group 2, whereas the AIx in Group 3 was higher than in either of these groups (Table 1).

All three groups exhibited a progressive decline in AIx between baseline and the development of hypoglycemia at R (Fig. 1). The fall in AIx from baseline to R was significantly less in Group 3 compared with the reduction in AIx from baseline to R in Groups 1 (*P* < 0.01) and 2 (*P* < 0.01). At R+30 min, AIx had risen to approach baseline levels in all three groups.

Changes in AIx, duration of insulin exposure, and insulin dose. There was no significant difference in the duration of the euglycemic clamp procedure or the time taken to induce hypoglycemia among the three groups. The duration of the euglycemic clamp [means (SD)] measured 37.3 (11.1) min in Group 1, 44.2 (8.5) min in Group 2, and 46.9 (10.0) min in Group 3 (*P* = 0.3). The time taken to induce the R measured 18.1 (6.4) min in Group 1, 22.4 (5.2) min in Group 2, and 20.7 (6.9) min in Group 3 (*P* = 0.5). However, among individuals within the separate groups, a correlation was observed between the time taken to induce hypoglycemia and changes in AIx, with a longer duration being associated with a greater reduction in AIx (Group 1, *r* = 0.25, *P* = 0.04; Group 2, *r* = 0.38, *P* = 0.03; Group 3, *r* = 0.28, *P* = 0.04). Similarly, no significant difference was found in the amount of insulin infused during the studies among the three different groups. The total insulin dose administered was 14.6 (4.4) U in Group 1, 12.7 (3.1) U in Group 2, and 15.7 (4.8) U in Group 3 (*P* = 0.6). However, within the separate groups, a significant correlation was present between changes in AIx and insulin exposure. A greater decrement in AIx was associated with greater insulin exposure (Group 1, *r* = 0.30, *P* = 0.05; Group 2, *r* = 0.34, *P* = 0.04; Group 3, *r* = 0.3, *P* = 0.03).

DISCUSSION

Arterial stiffness is increased in people with type 2 diabetes (5, 7, 16, 17), in their nondiabetic relatives (19), and in people with impaired glucose tolerance (37). In people with type 1 diabetes, discrepant results have been reported. Some studies

Table 1. Characteristics of participants in the study

	Group 1 (n = 10)	Group 2 (n = 10)	Group 3 (n = 10)	ANOVA
Age, yr	29.4 (4.4)	30.2 (7.9)	31.1 (4.7)	<i>P</i> = 0.8
Duration of diabetes, yr		3.0 (1.1)	18.4 (0.7)	<i>P</i> = 0.3
HbA1c		7.6 (0.7)	8.1 (0.3)	<i>P</i> = 0.2
Body mass index, kg/m ²	24.0 (1.2)	24.2 (1.1)	24.4 (1.4)	<i>P</i> = 0.9
Total plasma cholesterol, mmol/l	4.5 (0.8)	4.7 (0.6)	4.8 (0.6)	<i>P</i> = 0.4
Peripheral systolic BP, mmHg	121.7 (10.5)	123.3 (6.5)	125.9 (9.8)	<i>P</i> = 0.3
Peripheral diastolic BP, mmHg	65.6 (7.4)	69.4 (7.7)	72.7 (6.7)	<i>P</i> = 0.2
Mean arterial pressure, mmHg	92.4 (8.7)	87.5 (9.1)	92.5 (9.1)	<i>P</i> = 0.4
Central systolic BP, mmHg	114.4 (14.9)	110.9 (8.0)	121.5 (9.7)	<i>P</i> < 0.05
Central diastolic BP, mmHg	77.2 (10.5)	74.8 (8.4)	79.0 (8.7)	<i>P</i> = 0.6
Augmentation index*	9.3 (12.4)	10.0 (8.3)	21.8 (6.9)	<i>P</i> < 0.05

Results are expressed as means (SD). Group 1, nondiabetic subjects; Group 2, type 1 diabetes, <5 yr duration; Group 3, type 1 diabetes, >15 yr duration. BP, blood pressure; HbA1c, glycated hemoglobin (nondiabetic range 4.3–6.5%). *Measured as augmented pressure expressed as % pulse pressure. Post hoc *t*-test results are as follows. Central systolic pressure: Group 3 vs. Group 1, *P* = 0.04; Group 3 vs. Group 2, *P* = 0.047. Augmentation index: Group 3 vs. Group 1, *P* = 0.02; Group 3 vs. Group 2, *P* = 0.002.

Table 2. Hemodynamic measurements in study participants

	Group 1 (n = 10)	Group 2 (n = 10)	Group 3 (n = 10)
Peripheral systolic BP, mmHg			
EC	121.7 (10.5)	123.3 (6.5)	125.9 (9.8)
0	121.1 (9.7)	117.8 (9.4)	131.8 (11.2)
R	*143.5 (11.8)	*137.8 (9.8)	*144.5 (7.9)
R + 30 min	116.9 (11.0)	114.6 (12.2)	130.6 (8.1)
Peripheral diastolic BP, mmHg			
EC	65.6 (7.4)	69.4 (7.7)	72.7 (6.7)
0	68.1 (6.1)	68.7 (7.0)	72.5 (7.1)
R	*61.6 (6.5)	*63.6 (9.9)	*68.7 (3.6)
R + 30 min	66.5 (11.2)	67.4 (14.6)	71.1 (6.7)
Mean arterial pressure, mmHg			
EC	92.4 (8.7)	87.5 (9.1)	92.5 (9.1)
0	92.6 (6.5)	88.1 (8.1)	92.9 (8.8)
R	92.3 (7.8)	87.7 (7.9)	93.9 (11.2)
R + 30 min	92.3 (8.5)	87.43 (9.0)	89.6 (12.0)
Central systolic BP, mmHg			
EC	114.4 (14.9)	110.9 (8.0)	120.5 (9.2)
0	114.2 (12.6)	109.9 (7.2)	123.8 (6.4)
R	*110.8 (13.9)	*108.1 (7.0)	*118.0 (7.7)
R + 30 min	113.5 (5.6)	110.5 (9.2)	122.8 (7.8)
Central diastolic BP, mmHg			
EC	77.2 (10.5)	74.8 (8.4)	79.0 (8.7)
0	77.3 (11.1)	75.1 (7.4)	79.1 (8.1)
R	77.3 (10.4)	74.8 (8.8)	78.7 (8.9)
R + 30 min	76.9 (8.9)	74.4 (9.0)	78.9 (8.2)
Heart rate, beats/min			
EC	60.5 (11.7)	66.5 (7.1)	70.7 (7.8)
0	60.2 (11.1)	66.3 (9.0)	68.4 (10.1)
R	*76.7 (12.8)	*82.2 (8.4)	*79.0 (7.1)
R + 30 min	59.1 (10.2)	66.2 (9.7)	66.8 (8.3)
Autonomic symptom score			
EC	0.2 (0.2)	0.5 (0.4)	0.2 (0.1)
0	0.4 (0.6)	0.6 (0.3)	0.8 (0.6)
R	*11.2 (4.4)	*9.9 (5.1)	*7.9 (1.4)
R + 30 min	0.5 (0.3)	0.8 (0.8)	0.9 (1.1)

Results are expressed as means (SD). Group 1, nondiabetic subjects; Group 2, type 1 diabetes, <5 yr duration; Group 3, type 1 diabetes, >15 yr duration. EC, euglycemic clamp commenced; 0, start of insulin infusion to induce hypoglycemia; R, autonomic reaction. *Significant within-group difference ($P < 0.05$).

have demonstrated greater arterial stiffness compared with nondiabetic controls (4, 36, 46), whereas others have shown either no difference (23) or lower arterial stiffness (26). These differences may relate to the methodologies used and to patient selection, with participants differing in their duration of diabetes, age and gender, smoking status, peripheral blood pressure, serum cholesterol concentrations, and the presence or absence of microvascular disease.

In the present study, arterial stiffness and central arterial pressure were studied both in nondiabetic adults and in people with type 1 diabetes of differing duration, using radial artery pulse wave analysis and calculation of the AIx, and the subjects, all of whom were nonsmokers, were matched for age, gender, blood pressure, serum cholesterol, and body mass index. The results showed no difference in vessel wall stiffness or central arterial pressure between a group with well-controlled type 1 diabetes of short duration and a matched cohort

of healthy nondiabetic volunteers. However, the people with type 1 diabetes of longer duration had greater stiffness of the arterial vessel wall and an elevated central arterial pressure compared with nondiabetic controls. This suggests that the duration of exposure to chronic hyperglycemia influences vessel wall stiffness rather than the presence of type 1 diabetes itself. This may result from structural changes caused by the gradual deposition of advanced glycation end products within the walls of large blood vessels (18). The endothelium (22, 50) and arterial wall smooth muscle bulk and tone (2) (the latter under the control of the endothelium) also influence the elasticity of arteries, and it is also possible, therefore, that increased vessel wall stiffness in people with type 1 diabetes of long duration is the result of functional changes that occur within the endothelium with increasing duration of diabetes.

In the present study, the rise in peripheral systolic blood pressure that is known to accompany acute insulin-induced hypoglycemia was accompanied by a reduction of central systolic blood pressure. These findings highlight the potential for divergent responses of central and peripheral arterial pressures and support the premise that the measurement of peripheral blood pressure by conventional methods does not provide an accurate measure of the central arterial pressure load on the left ventricle. Specifically, the results show how the changes of central and peripheral arterial pressures differ in response to the stress provoked by acute hypoglycemia and mediated by sympathoadrenal stimulation. It could be speculated that the reduction in central arterial pressure that was observed during acute hypoglycemia may lead to a reduction of coronary perfusion, which could potentially precipitate a vascular event in people with vascular disease.

The difference that was observed between the responses of peripheral and central systolic blood pressures to insulin-induced hypoglycemia is indicated by the observed changes in AIx; a reduction in AIx reflects diminished amplification of the systolic pressure wave and so causes a net overall reduction in central arterial systolic blood pressure. It is possible that this observed change in AIx had resulted either from changes in heart rate (45) or from the actions of vasoactive hormones that are secreted in response to hypoglycemia (38), or as a combination of both effects. An increment in heart rate of 10 beats/min causes a fall in AIx of ~4% (45), and the infusion of

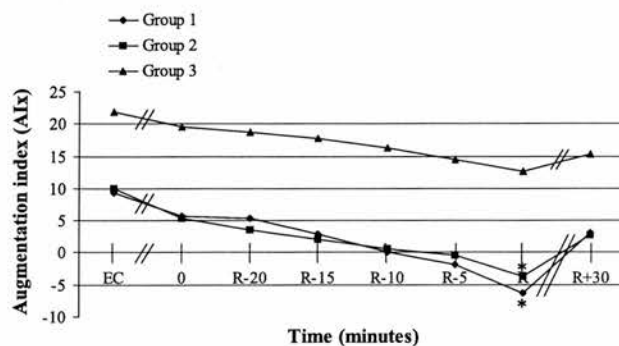


Fig. 1. Mean change in augmentation index (AIx). Group 1, nondiabetic subjects; Group 2, type 1 diabetes, <5 yr duration; Group 3, type 1 diabetes, >15 yr duration. EC, euglycemic clamp commenced; 0, start of insulin infusion to induce hypoglycemia; R, autonomic reaction; R-20, R-15, R-10, and R-5, 20, 15, 10, and 5 min before R, respectively; R+30, 30 min after R. * $P < 0.0001$ compared with values at 0.

norepinephrine promotes an increase in AIx (48). However, in the present study, the AIx initially was observed to fall during euglycemia, before any stimulated changes in heart rate, autonomic symptom score, or peripheral blood pressure. Insulin per se may have a direct effect on the functional integrity of large blood vessels. In the present study, no significant differences were observed in the duration of insulin exposure or in the total insulin dose administered, but it was observed that, in individuals within each study group, a significant correlation existed between changes in AIx and the duration of insulin exposure and, consequently, the amount of insulin infused. This would support the suggestion that the early changes in AIx may therefore be a consequence of the direct action of insulin on endothelial function or on smooth muscle tone of large arteries. This interpretation would be consistent with the results of other studies that have demonstrated that the intravenous administration of insulin increases the distensibility of large arteries (39, 40).

The decrement of AIx was less in the group with type 1 diabetes of longer duration compared with that of subjects with diabetes of short duration and nondiabetic subjects. This would be consistent with greater stiffness and diminished elasticity of the vessel walls of large arteries in people with type 1 diabetes of long duration. Consistent with this observation, Westerbacka et al. (41) have shown that the effect of insulin to reduce vessel wall stiffness in large arteries is diminished in people with type 1 diabetes who have no vascular complications. The mean duration of type 1 diabetes in their participants was 18 yr (41), which is similar to the duration of diabetes of Group 3 in the present study. It has to be acknowledged that the algorithm to relate peripheral and central pressures by applanation tonometry was derived in nondiabetic subjects. However, in the present study, the changes in the central hemodynamic responses to insulin infusion and the induction of acute hypoglycemia were similar in both the nondiabetic and the diabetic groups.

Type 1 diabetes is associated with an increased prevalence of cardiovascular disease, and vessel wall stiffness is an independent predictor of cardiovascular morbidity and mortality. The present study has demonstrated that a greater duration of type 1 diabetes was associated with an increase in vessel wall stiffness, before the development of overt macrovascular disease. Central systolic pressure at rest was also greater in people with type 1 diabetes of longer duration, although peripheral blood pressure did not differ among the three study groups. Changes in brachial pressure may therefore underestimate changes in aortic pulse pressure and the response of left ventricular systolic function to hemodynamic stress in people who have had type 1 diabetes for several years. These vascular changes and the attendant rise in central arterial blood pressure may contribute to the increased risk of cardiovascular morbidity in people with type 1 diabetes.

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DISCLOSURES

There are no conflicts of interest in this work.

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